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THE NORMAL AND PATHOLOGICAL HISTOLOGY OF THE VENTRICULUS OF THE HONEY-BEE, WITH SPECIAL REFERENCE TO INFECTION WITH *NOSEMA APIS*

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In the great amount of work which has been done on the organisms of insect-borne diseases and on micro-organisms found as parasites in insect tissues, the pathological histology of the insect itself has been singularly neglected. Furthermore it is found that the basis for such study—namely, a knowledge of the normal histology and cytology—is often almost entirely lacking. The fact that many of the intracellular symbionts of insects and the granule-like Rickettsia organisms of typhus and trench fever, were for some time after their discovery confused with normal cell inclusions, well illustrates the need for study of the normal histology and particularly of the granular cell inclusions universally present in insect tissues. While the primary purpose of this paper is a consideration of the pathology of the adult honey-bee, there has necessarily been included as a basis therefor, a somewhat extended discussion of the normal histology and cytology. This broader phase of the problem it is believed will appeal to the parasitologist or other worker with micro-organisms, quite as strongly as the pathology itself.

THE DISEASES OF ADULT HONEY-BEES

The diseases of bees best known to the beekeeper, and those ordinarily referred to under the term "bee diseases," especially in America, are the diseases of the larvae or brood. Of these the most common and the most serious are the bacterial diseases, American foulbrood and European foulbrood. In addition to the larval diseases, there are a great number of disorders of the adult honey-bee, accounts of which are found in beekeeping literature as far back as three hundred years. (Bullamore 1922; Zander 1911, 1921.) These disorders, nearly all of obscure etiology, are characterized by a variety of symptoms and are known to the beekeeper under a wide variety of names. Besides those conditions supposedly due to old age, exposure, insufficient or improper food, or poisoning, may be mentioned dysentery, diarrhoea, Ruhr, May-sickness, May-pest, June-sickness, paralysis, palsy, trembling, dizziness,

vertigo, spring dwindling, disappearing disease, dropsy, Sandläuferei, Fussgängerei, Isle of Wight disease, Nosema-disease, Aspergillusmycosis and "paratyphoid." The variety of symptoms exhibited in these disorders is indicated to some extent by their names. A review of the literature reveals the very greatest confusion as to nomenclature, the symptoms associated with a given disease, and etiology. Despite the multiplicity of names and the variety of symptoms, these disorders are all marked by one outstanding feature—namely: the affected bees, unable to fly, are found crawling or nearly motionless, usually near the hive, where they die after a few hours.

While these disorders may occur at any time of the year, they are most common in late winter and early spring, being known frequently as winter losses, spring dwindling, May-sickness, etc. They appear to be highly contagious in some cases and in others not at all so. There is no constant correlation of any of the disorders with such external factors as periods of unfavorable weather or the blossoming of certain nectar- or pollen-producing plants.

It is probable that many of the disorders which have been considered distinct pathological entities are in reality only symptoms of diseases which may be evidenced externally by a number of different conditions. This is well illustrated in the literature of Isle of Wight disease and of Nosema-disease, the best known of the diseases of adult bees, in which the external symptoms of nearly all the various disorders have been described at one time or another as characteristic of each of these two diseases. Indeed it would appear that the various symptoms are not specific for any disease but are evident whenever the bees are weakened from any cause. The causes of such weakening are not known except in the few cases where definite organisms have been described. The stricken bees may exhibit marked abdominal distention with copious defecation on combs, hive or ground, this condition being known as dysentery or Ruhr. In "dry dysentery" or "constipation," there may be abdominal distention with apparently no defecation. The feces may be thin and watery, or thick and ropy, light or dark in color and acrid or otherwise in odor. The bees appear weakened, and are found mostly on the alighting board or on the ground in front of the hive, singly or in groups, clinging to spears of grass, lying nearly motionless on the ground, or crawling about either actively or sluggishly. The bees are unable to fly, though they may leave the ground for a flight of a few inches, their progress being then a combination of active crawling and "hopping." At times certain of the legs seem to be paralyzed and are dragged along in crawling. The wings are often "dislocated," i. e., the hind wings are not hooked to the fore wings, and are capable of only an irregular trembling motion, or at best a feeble, jerky fanning.

In crawling, the bee may hold itself as if bent to one side, and may describe small circles. The terms paralysis, trembling, palsy, vertigo, etc., are used to designate these various conditions.

Nosema-disease (Nosemaseuche) is the term proposed by Zander (1911) to designate an infectious form of Ruhr (dysentery) and Maikrankheit (May-sickness), with both of which he found associated the Microsporidian, *Nosema apis,* described by him in 1909. Zander considered copious defecation the surest sign of Nosema-disease, though this symptom was frequently absent. The sudden appearance of groups of bees dying inside or outside the hive was held to be an important characteristic. White (1919) studied Nosema-disease in America and concluded that it is an infectious, though not particularly malignant disorder of the adult bees. The parasite is cosmopolitan in its distribution. In heavy infections the colonies become weak and may be destroyed. The behavior of the infected colony is similar to that of a healthy one. The individual infected bees manifest no external symptoms until actually dying, when they become unable to fly and crawl about as described above. Diagnosis of the disease is confirmed by finding the spores of the parasite in the ventriculus.

Isle of Wight disease, named from the epidemic which was first reported from the Isle of Wight in 1906, has caused great losses in the British Isles. The disease was first described as "paralysis" and "dysentery" from the symptoms most commonly observed. However, it was soon shown that these symptoms were not invariably present. Indeed the only constant symptoms were the inability of the stricken bees to fly and their ultimate death. Fantham and Porter (1912a, 1912b) in their studies on Isle of Wight disease found *Nosema apis* in more or less constant association with the disease, and together with other British workers, considered this organism to be the cause of Isle of Wight disease (Graham-Smith, Fantham et al., 1912). Rennie and Harvey (1919a, 1919b), however, while holding Isle of Wight disease to be infectious, concluded that *Nosema apis* was not causally related thereto. This has apparently been confirmed by the work of Rennie, White and Harvey (1921) who found the thoracic tracheae of diseased bees containing numbers of the mite *Tarsonemus woodi,* which they considered the cause of Isle of Wight disease. These workers have also discussed the relation of the mite to the pathology of the disease.

A number of other presumably infectious disorders of adult bees associated with micro-organisms, or otherwise, may be mentioned. In Brazilian bee-pest the bees die in great numbers as in Isle of Wight disease and Nosema-disease. While the disease has been attributed to poisonous nectar, the cause remains unknown (Zander 1911). Maassen (1916a) mentioned Aspergillusmycosis, a disease affecting both larvae and adults, caused by a species of Aspergillus. Zander (1911, 1921)

and Maassen (1919) both mentioned the larvae of a Meloid beetle, *Meloë variegatus,* as at times causing losses of adult bees. The meloid larvae are found attached to the intersegmental membranes of the abdomen, having been picked up by the bees in foraging. Maassen (1919) also reported the finding of an ameba-like organism in the Malpighian tubes of adult bees, which in one case at least was associated with the death of many bees. Bahr (1919) found a bacillus of the paratyphoid group in the intestine of adult bees which were unable to fly and which were dying in numbers. Feeding pure cultures of this organism reproduced the disease. Nosema was not found in connection with this disorder.

In America and Australia serious losses of adult bees have occurred for which no satisfactory cause could be assigned, the symptoms being similar to those described for Isle of Wight disease and Nosema-disease. *Nosema apis* is known to occur in these countries and may be partly responsible for this damage, though the presence of Nosema has not been demonstrated in all cases.

It is thus seen that there are a number of disorders in which the adult bees die in numbers, exhibiting a variety of symptoms. In some cases organisms are known to be associated with, and are perhaps the cause of, the disease. In others the cause can not be designated with certainty. None of these disorders is characterized by any outward symptom definitely diagnostic for that disease. In all cases the stricken bees are unable to fly and die in numbers. It is possible, as suggested by several investigators, that inability of the bees to fly and various other conditions accompanying their death and cited as symptoms of different diseases, may represent merely the final stage in the weakening of the bees due to whatever cause.

It would accordingly be possible to have any number of disorders of adult bees, or rather disorders due to any number of different causes, in which the outward symptoms would be the same. The evidence already cited seems to indicate that such is the case. Since external symptoms are nearly valueless in indicating the real nature and causes of diseases of adult bees, the study of internal pathological conditions would seem to promise the best basis for accurate diagnosis. This method has been pursued in certain bee diseases with which an organism is definitely associated, as in Nosema-disease. The emphasis, however, has been placed on determining the presence or absence of the parasite rather than on the condition produced in the host tissues. Even here the possibility of factors other than the parasite contributing to the pathological condition is not precluded. The fact that many stricken bees contain but a few parasites, while other apparently healthy bees are heavily infected, indicates that other factors do operate in Nosema-disease. If analysis of factors other than the known or suspected

organism is necessary in disorders where such an organism has actually been demonstrated, the need for such study is all the more imperative in those disorders of adult bees of which the cause is wholly unknown or in those which have been attributed to such indefinite factors as long winter confinement, bad weather, and poisonous pollen or nectar. A comparative study of the histology and cytology of both healthy and diseased bees would seem to be one of the most promising methods of attacking the problem.

No group of animals furnishes examples of parasitism greater in point of number and diversity of nature than the insects and other arthropods. Nevertheless most of our knowledge of these parasites concerns only the organism itself, singularly little being known of the effect of the parasite on the host. In many cases in which a parasite causes a disease of economic importance the disease itself is well understood as concerns symptoms, the causal organism, the manner of transmission, and the methods of control, while little attention has been given to the pathological histology of the host. Beyond certain of the grosser and more obvious features, the changes brought about by the parasite in the host cells and tissues and the disturbances of their functions are almost unknown.

This lack of knowledge of pathological histology is especially marked in connection with diseases of the adult honey-bee, in which the diseased conditions themselves are not well understood. As a preliminary investigation of the pathology of the honey-bee from the histological point of view the present study was undertaken with the purpose of determining what changes are brought about in the tissues and cells of the ventriculus by the presence of *Nosema apis*, and further of determining what pathological conditions this organ may exhibit in certain disorders not associated with the presence of *Nosema apis*.

MATERIALS AND METHODS

Material was obtained chiefly from the University apiary, University Farm, St. Paul, during the years 1916-17 and 1919-21, and from Cornell University apiary at Ithaca, 1917-18. Bees were taken mostly from the hive entrance, though occasionally from within the hive or from blossoms. The bees were dissected as soon after their capture as possible, usually within an hour or two. After the head was cut off, the entire digestive tract was withdrawn by seizing the tip of the abdomen with forceps and pulling gently. The ventriculus was separated from the hind-intestine and dropped into 0.75 per cent. sodium chloride solution or into Locke's solution. Another method of obtaining the ventriculus was to cut off the entire abdomen and drop it into physiological saline solution. With fine forceps sufficient of the

chitinous covering was torn away to enable the ventriculus to be removed easily. Live bees were used in all cases in order to avoid any possible effects of chloroform or other killing agent.

In examining the ventriculus in the fresh condition, the whole or a small portion of the organ was crushed beneath a cover-glass. The best results were obtained by using small portions prepared as follows: A small section, about one-half a millimeter in thickness, was removed by means of scissors, this section appearing as a small white ring (the ventriculus wall) with a brownish gelatinous mass (the peritrophic membranes) within. This brownish mass was removed entire with forceps, leaving the wall intact; or the wall itself was cut with scissors, whereupon it would turn inside out, separating itself almost entirely from the peritrophic membranes. In this manner a portion of the wall of the ventriculus could be mounted free from its contents. Hanging drops were also made by touching the cut end of the ventriculus to a cover-glass and inverting this over a hollow-ground slide. The latter method allowed prolonged examination without changes in location or appearance of the material due to drying.

Cover-glass preparations were made usually as shallow hanging drops which were observed in the fresh condition and then fixed by being dropped flat upon the surface of sublimate-alcohol, or placed over a Van Tieghem cell containing 2 per cent. osmic acid solution, and then hardened by dropping upon 70 per cent. alcohol. By the latter method the preparation could be watched during fixation. Thus by noting its location a given cell could be studied in the fresh condition, during fixation and after staining.

Sections 3 to 5μ thick were made from material fixed in sublimate-alcohol, Bouin's fluid, Zenker's fluid, Gilson's mercuro-nitric solution, Hollande's (1912) bichromate-formol solution and in osmic acid vapor. The stains used for sections were mostly Heidenhain's iron-hematoxylin, counterstained with eosin, Delafield's hematoxylin, and Romanowsky stain.* For cover-glass preparations the most successful stains were Giemsa (Gruebler's Giemsalösung) and Romanowsky.* The preparations were left in the stain from 15 minutes to 24 hours, washed in distilled water, dehydrated rapidly in 95 per cent. alcohol or in acetone, passed through xylol and mounted in balsam or cedar oil.

THE NORMAL VENTRICULUS

Determination of the changes produced in the ventriculus by the presence of parasites, or the histological diagnosis of any other pathological condition, must be preceded by consideration of the normal

*"Romanowsky" stain, obtained from Noyes Brothers & Cutler, St. Paul, prior to 1915: equal portions of two stock solutions, "eosin" solution and "methylene blue" solution, diluted 1 to 10 in distilled water.

ventriculus, or rather the range of different conditions exhibited by this organ taken from apparently healthy bees. The criterion by which a bee is to be judged "healthy" or otherwise, is necessarily indefinite, since bees heavily infected with parasites may be outwardly indistinguishable from uninfected bees, while other bees may die in numbers from no apparent cause. Until more exact information is available concerning disorders of adult bees, "healthy" bees may be considered those in which neither external nor internal pathological conditions are apparent and which are obtained from a strong, vigorous colony, or at least one not suffering any marked loss of bees.

The ventriculus, or mid-intestine, lies in the anterior and dorsal portion of the abdomen. It is a more or less U-shaped tube, of uniform diameter, with numerous annular constrictions. It is ordinarily yellowish or whitish translucent in appearance, with reddish brown contents which give the whole a reddish brown color, though this varies somewhat from pale yellowish brown to dark brown. A deep constriction, the proventriculus, separates the ventriculus at its anterior end from the crop or honey-sac. About the ventriculus is the tangled mass of whitish or yellowish Malpighian tubes which empty into the digestive tract at the junction of the ventriculus and the small intestine, the latter being a coiled tube of about one-third the diameter of the ventriculus.

The structure of the ventriculus has been described by a number of workers (Frenzel 1886, Snodgrass 1910, White 1919, Pavlovsky and Zarin 1922). The wall of the ventriculus is made up of a number of structures. Forming a network on the outer or coelomic surface are three layers of muscle fibers, the outer and inner layers being longitudinal and the middle layer transverse. Within the muscle layers is the basement membrane which is continuous anteriorly with that of the fore-intestine (esophageal valve) and posteriorly with that of the Malpighian tubes and small intestine. Attached to the basement membrane are the cells composing the epithelium. The epithelial cells possess many fine, more or less parallel hairs arising from their surface and extending into the lumen of the ventriculus, these constituting the striated border. In the lumen and extending the entire length of the ventriculus are the peritrophic membranes, concentrically arranged. The outer, and hence most recently formed, peritrophic membrane either lies free in the lumen, sharply differentiated from the epithelium, or merges gradually with the substance of the striated border.

The inner surface of the epithelium is not regular, but appears in section to be studded with projections or villi, between which are pits or crypts (Fig. 1). The projections are joined to each other forming a honey-comb of walls surrounding the cylindrical crypts. At the bottom of the crypts are the nidi composed of the many small regen-

eration cells, while the walls are composed of columnar or elongate-pyriform cells. Each of the latter is attached to the basement membrane by a narrow stalk, while the free end tends to be as nearly spherical as the surrounding cells permit. The total thickness of the epithelium averages approximately 70μ, with extremes of 30 and 110μ. At the bottom of the crypts the epithelium measures about 25μ in thickness.

The striated border forms a layer lining the inner surface of the epithelium and more or less completely filling the crypts. That this layer is made up for the most part of many fine hairs, is seen in sections of material fixed in various solutions. In addition the hairs may be demonstrated in almost every fresh mount and are striking in appearance. They radiate from one side or at times apparently from the entire periphery of the isolated cells (Figs. 10, 18-21).

Lying over the striated border and uniting the free ends of the hairs extends a membrane of varying thickness and somewhat indefinite outline, staining like the substance of the striated border, though more intensely, as if it were a condensation of that substance (Figs. 1, 3, 6 and 8). This membrane is apparently the peritrophic membrane in process of formation, since it may frequently be demonstrated to be continuous with a well defined peritrophic membrane which has become detached from the epithelium in another portion of the ventriculus. While a number of workers have considered the peritrophic membranes to be the product of certain cells at the anterior end of the mid-intestine (see discussion in Deegener, 1913), it would appear from the writer's observations which in general agree with those of Pavlovsky and Zarin (1922) that the peritrophic membranes of the honey-bee are formed from the substance of the striated border, together with the remains of cells which are given off from the epithelium in the process of digestion, and that this formation takes place not directly at the surface of the cells but at the free ends of the hairs forming the striated border. Snodgrass (1910) does not mention the hairs, but figures an "intima" with a "gelatinous mass" between the intima and the epithelium, the gelatinous mass apparently representing the striated border. The "intima" or "cuticle" (Pavlovsky and Zarin) together with the striated border separate from the epithelial cells, shrivel and shrink and finally become peritrophic membranes. The process is repeated in the formation of each successive membrane. These lie one within another, appearing in section as thin, much wrinkled bands, somewhat refractive, sharply defined in some regions, in others merging with a finely granular substance containing here and there remains of epithelial cells. The peritrophic membranes stain slightly or not at all, being usually yellowish in color, while the granular masses associated with them stain with eosin. In the fresh condition the peritrophic membranes are seen as a reddish brown, gelatinous mass, which on

traction with forceps separates easily from the epithelium. The innermost membrane forms a tube for the passage of pollen grains and other food, and at times portions of it pass down with the food and are found in the hind-intestine surrounding little pellets of pollen.

A long tubular fold of the fore-intestine projects into the lumen of the ventriculus, forming the esophageal valve. The basement membrane bears a layer of epithelial cells somewhat smaller than those of the ventriculus. Many of these cells are stalked like those of the ventriculus, and appear to be given off from the epithelial layer from time to time. While there is no sharp dividing line between esophageal valve and ventriculus, the cytoplasm of the ventriculus cells is markedly more deeply stained than that of the valve cells.

The contents of the ventriculus during the field season usually consist only of the peritrophic membranes and the gelatinous substance associated with them. A few pollen grains are found at times, though these apparently pass rapidly to the hind-intestine where they may be found in great numbers in all stages of digestion. When the bees are in winter quarters, or at times when cleansing flights are infrequent, the ventriculus may contain a considerable accumulation of pollen, particularly at the posterior end, together with feathered bee hairs and many micro-organisms, chiefly bacteria and yeasts. The solid portions of the contents are usually enclosed by the inner peritrophic membrane, there being no direct contact with the epithelium. Occasionally, however, there may be found in sections small groups of bacteria lying next the epithelium. Whether or not bacteria can make their way through several uninjured peritrophic membranes is not known.

The intestinal flora of the honey-bee includes a number of different bacteria and yeasts, some of which are almost constantly present (White 1906). The greater number of these organisms are found in the hind-intestine, particularly the rectum, relatively few both as to numbers and variety being found at any time in the ventriculus, except at those times when an accumulation of food material occurs as noted above. The great difference in bacterial content between mid- and hind-intestine is noticeable at once in fresh preparations, and is confirmed by broth and agar cultures. The writer has at times obtained no growth in media inoculated with a small section of wall and contents of the ventriculus, while very frequently no growth resulted in tubes inoculated with a small section of the ventriculus wall freed from the peritrophic membranes. The explanation for the slight bacterial content of the ventriculus lies perhaps in the fact that solid particles pass rapidly to the hind-intestine, and further that the contents of the ventriculus are at times rather acid, which may inhibit multiplication of organisms.

One of the most striking features encountered in examining the epithelium of the ventriculus is the extreme variation of the cells and

their nuclei as to size, shape and arrangement, and as to the character, size and number of the cytoplasmic inclusions. These variations are due not only to differences in location in the epithelium and to differences in age, as are found for example in the regeneration cells and the cells of the crypt walls, but probably also to differences in function in the process of digestion, not as yet well understood. It is correspondingly difficult, therefore, to determine just what cells are to be considered "normal" and those which may exhibit some pathological condition.

In the bee, as in most insects, cells or portions of cells are given off into the lumen of the ventriculus, these being known as secretion cells (Frenzel 1886) or sphaerocytes (Deegener 1913). These apparently contain digestive enzymes, which are liberated in the lumen of the ventriculus by the disintegration of the cells. Pavlovsky and Zarin (1922) have discussed the ferments of the digestive tract of the bee. The proliferation of the secretion cells takes place chiefly from the walls of the crypts and rarely from the nidi at the bottom. Snodgrass (1910) stated that these "discharged cellules" all contain nuclei, and believed their proliferation to be due to an active division of cells forming the walls and lips of the crypts. According to the writer's observations the secretion cells may arise in several ways:

1. In some cases it appears that the entire cell, the basal portion of which usually consists of a slender stalk, elongates greatly and finally separates completely from the basement membrane. Such a secretion cell at first differs from cells of the epithelium only in that it has become nearly spherical. This condition is seen occasionally in sections in regions where the epithelium is greatly thickened as a result of the elongation of the cells. That the individual cells are easily detached from the basement membrane is shown by the great number of large, spherical, nucleate cells, apparently uninjured, to be found in every fresh preparation (Fig. 10).

2. In both sections and fresh mounts, especially the latter, are always to be observed many spherical secretion cells without nuclei, which average somewhat smaller than the nucleate cells just described (Figs. 11-20). These may arise in several ways: A division of the cell body without nuclear division may take place, though this has not been observed. In fresh mounts there have been noted frequently, however, two or more cells, equal or unequal in size, joined together as shown in Figures 17 and 18. What is apparently the same condition has been observed in sections of the normal ventriculus undergoing great proliferation of secretion cells. Many elongate secretion cells bear on their distal extremities another secretion cell. Not uncommonly there are several cross-walls in the latter, indicating perhaps as many different stages in their formation. Whether a

division of the parent cell takes place or whether one cell is formed on the surface of the other by a process resulting in what may be compared to a blister, cannot be stated. The latter method seems probable from the fact that very frequently the "blister" is clear and homogeneous while the parent cell may contain a nucleus together with the characteristic granules described below. Both may contain such granules, however. That actual division of the parent cell does not take place is further shown in sections of the ventriculus infected with Nosema, in which the same or a similar process may at times be noted. Figure 7 shows several of the greatly elongate epithelial cells bearing on their free ends tiny cap-like cells, several of the latter containing cross-walls similar to those observed in uninfected specimens. From the fact that these tiny secretion cells contain no spores and that their cytoplasm stains much more intensely than that of the infected parent cells, it is evident that no division of the parent cell body has taken place, but rather that the cap-like cells have obtained the material of which they are composed through osmosis.

3. In nearly all fresh preparations occur isolated cells beraing a stalk. These vary in size from tiny cells a few micra in diameter to those as large as the average epithelial cell. They are without nuclei, their cytoplasm may contain the refractive granules characteristic of the epithelial cells, and the hairs of the striated border are usually, though not always, present (Figs. 19, 20). Occasionally a tiny cell of this sort is seen attached by its stalk to a larger cell which may also be stalked. As many as three or four tiny cells may be joined by their stalks, forming a chain. These recall the sphaerocytes described and figured by Deegener (1913) from the mid-intestine of the larva of *Deilephila euphorbiae*. In the honey-bee they seem to arise through a constricting off of the surface portion of the epithelial cells. The number of these stalked secretion cells varies greatly in different specimens; in some they are rare or absent while in others certain parts of the ventriculus are lined with them. The rôle which these various types of secretion cells play in the digestive processes, and the conditions which give rise to one type or another, are not understood.

The nuclei vary greatly in appearance, both in the fresh and stained condition. They are spherical, oval or rarely elongate. The nuclei of the nidi as seen singly or in groups in fresh preparations are spherical and resemble fused masses of small, more or less spherical, non-refractive granules. Frequently there are also to be observed in the nucleus one to several small masses, very slightly refractive, either spheroid or irregular in shape. The nuclei of the nidi stain more intensely on the whole than do those of the surrounding cells, and appear either as a mass of small spheroid granules, intensely stained,

or as a lightly stained granular area containing one to several large, deeply stained granules or masses with definite outlines. These latter apparently correspond to the slightly refractive masses seen in the fresh condition. Though more or less active division of these cells undoubtedly takes place, nuclei in division are rarely seen, either in fresh or stained material. Two nuclei are to be observed in cells occasionally, however. Figure 2 represents a regeneration cell nucleus with division, apparently by amitosis, nearly complete.

The nuclei of the cells forming the crypt walls when freshly dissected usually appear as spheres of a clear liquid in which are suspended a number of slightly refractive masses, spheroid or somewhat elongate. These masses may be few and comparatively large or small and very numerous. The clear body of the nucleus, which at times is not homogeneous but is indistinctly granular, may or may not be liquid. However, the suspended masses appear to be firmly held, since they never exhibit Brownian movement as do the granules in the cytoplasm. The nucleus seems to be less easily destroyed by mechanical means than the rest of the cell, since many isolated nuclei, quite intact, are to be found in fresh mounts. The appearance of the nucleus fixed in osmic acid vapor is generally little changed from that of the fresh nucleus, the body of the nucleus being homogeneous or finely granular, and staining lightly, while the suspended masses stain deeply (Fig. 3). Nuclei fixed by methods other than osmic vapor in general appear either coarsely granular or coarsely reticulate (Fig. 1). Lewis and Lewis (1915) obtained similar results in fixing chick embryo cells, finding that only with osmic acid vapor was the appearance of the fresh nucleus faithfully preserved, while other fixers yielded the reticulate "text-book" nucleus.

Another condition of the nucleus is often to be observed in any one of the different types of epithelial cells, in both fresh and stained preparations. The nucleus is spherical or oval and contains what appears to be a clear homogeneous liquid. Suspended in this liquid or lying at one side of the nucleus, is a dense, granular, spheroid or irregular mass, the granules being slightly refractive with indistinct outlines (Figs. 10, 22, 23, 25). When stained such nuclei appear as vacuoles containing a densely stained, granular body.

THE CYTOPLASM AND ITS INCLUSIONS

The cytoplasm itself, whether of the nucleate epithelial cells or of the discharged secretion cells, is generally a clear, colorless liquid, though at times it is indistinctly finely granular. Its liquid or semi-liquid condition together with the very thin, elastic cell membrane, allows the cell to assume almost any shape in response to pressure of its neighbors, the cell becoming spherical when the pressure is removed.

In the cytoplasm are suspended the refractive granules or other inclusions, these being frequently in active Brownian movement. In sections the cytoplasm appears finely granular. It may be evenly distributed throughout the cell, or may be gathered into fine, irregular strands forming a network. In cells fixed in osmic acid vapor occur tiny vacuoles representing the spherical granules which are destroyed in the process of preparing paraffin sections. With other fixers it is but rarely that any trace of the spherical granules, which are so conspicuous in the fresh condition, is found in the sections.

The cytoplasmic inclusions are among the most puzzling features of the epithelium. Chief among these are the highly refractive, spherical granules which are found in nearly every cell. Though granules resembling those of the honey-bee occur in the mid-intestinal epithelium of nearly all insects, the information to be found in the literature concerning such granules, is rather meager. In most descriptions of the intestine of various insects, these granules, where mentioned at all, are referred to as secretion granules or fat droplets, with no further discussion. Frenzel (1886) in his work on the mid-intestinal epithelium of a number of insects, including the honey-bee, has described and figured "Sekretkügeln" or "Safttröpfchen" found in the cytoplasm. They have also been noted by Petersen (1912). Koehler (1920) has considered in some detail the nature of certain of these bodies and their possible relation to digestion.

The cytoplasmic inclusions may be divided into several groups: (1) spherical, refractive granules, 1 to 3μ in diameter; (2) very tiny, refractive granules, spherical or somewhat irregular, 0.2 to 0.5μ in diameter; (3) irregular, slightly refractive bodies; (4) faint, non-refractive rods; (5) vacuoles. These groups are considered below:

1. Koehler (1920) in her work on the inclusions of the epithelial cells of the honey-bee's intestine and the related problems of digestion, discussed but one type of inclusion, the spherical, refractive granules measuring 1 to 3μ. These conspicuous bodies are found in greater or less numbers in almost every cell. They have been variously supposed to be secretion granules playing some rôle in digestion (Zander 1911), or reserve food material (Frenzel 1886). Koehler, as a result of various staining reactions and other microchemical tests, concluded that the granules are composed of some calcium compound, probably calcium carbonate, with an outer covering of some organic material. She believed they might indicate an excretion of calcium by the epithelium, or that they might play an important rôle in the neutralization of acids formed in digestion. These bodies may be found in any cell of the epithelium though the regeneration cells contain few or none (Figs. 10-12, 14, 16-23). The number to be found in any cell varies greatly. In some specimens almost every cell in the epithelium is

literally packed with granules, while in others there may be many cells which contain only a few or none at all, as is the case with many secretion cells (Figs. 13, 15).

The granules are perfectly spherical, appear quite homogeneous, and are highly refractive. They are hyaline, appearing under the microscope bluish or greenish by daylight. They greatly resemble droplets of fat. They are distributed throughout the cytoplasm of the cells and are frequently in active Brownian movement, though after standing some time they usually sink to the bottom of the cell, remaining there in a mass. The granules occur mostly as single spheres, though a great many of these are in pairs, clinging together in spite of Brownian movement or of violent currents in the preparation. Granules in such pairs appear slightly flattened at point of contact. This flattening is a refraction phenomenon, and is also seen when two granules accidentally come together, though it strongly suggests a division form, especially in view of the great number of such pairs which even on prolonged observation are almost never seen to become separated. In this connection it may be mentioned that there have been seen on several occasions forms as shown in Figure 27, the granules being elongate with a constriction at the middle. In such granules the material composing the two halves is seen to be continuous. While such forms are very rare in the honey-bee, in another of the Hymenoptera, *Halictus* sp., one specimen of which was examined, similar granules were found, and in nearly every epithelial cell, one to several such double granules were noted. The significance of these forms is not known. Hanging drops of epithelial cells and isolated granules in various solutions and culture media have been observed for periods of days, but no division or multiplication has ever been seen to take place. When the granules are allowed to stand in Locke's or other saline solution, a marked change in appearance usually occurs after a short time. The refractivity becomes somewhat lessened and the granules assume the appearance of hollow spheres containing a clear liquid or other substance (Fig. 29). This appearance is characteristic for the most part of the smaller granules. The outer covering of the larger ones frequently appears to be double (Figs. 28, 30).

The writer's experience confirms that of Koehler as concerns the failure of the granules to stain with most of the common histological stains. However, with Romanowsky stain, employing wet films fixed in osmic acid vapor and hardened in 70 per cent. alcohol, it is almost always possible to obtain preparations in which the granules appear embedded in the blue cytoplasm like perfectly clear, sharply defined vacuoles, containing each a deeply stained, blue-purple body, about half the diameter of the vacuole (Fig. 9). Similar results were obtained

with Giemsa. The staining even in the same mount, is frequently very irregular, for causes not understood. The deeply stained inner body is usually noted in those granules which are contained within an epithelial cell, while those isolated frequently lack the inner body. In general, the inner body retains the stain tenaciously throughout differentiation and dehydration in acetone or alcohol. Very rarely the entire granule stains solidly, or appears unstained in the center with a film of stain over the surface. The inner body is rarely at the center of the granule, but almost always lies touching one side. The problem of the nature of the granules remains unsolved. Nothing whatever is known of the method of their formation. Is the material deposited in this form by the cytoplasm, somewhat as crystals are formed, or does the organic portion of each granule, if such there be, bring about its formation after the fashion of plastids in plant cells? Are such organic portions self-reproducing or is each formed *de novo* by the cytoplasm? These and related questions can be answered only after much further study.

2. In addition to the larger granules just described, are frequently found very tiny refractive granules, spherical or somewhat irregular in shape, and measuring 0.2 to 0.5μ in diameter (Figs. 10, 12, 16-19, 24-26). These closely resemble the larger granules in appearance except for the irregularity in form. Even when irregular, they are approximately spherical. Rod-like forms are not uncommon and groups have been noted in which nearly all the granules were tiny rods, in length approximately one and one-half times the diameter. These small granules occur singly and in pairs. As is to be expected on account of their small size, they are in much more active Brownian movement than the larger granules, and are usually to be found actively dancing about long after the others have sunk to the bottom of the cell. The tiny granules have been considered as being distinct from the larger ones for several reasons, namely: their irregularity of form; the presence of rod-like forms; the fact that they are all of approximately the same diameter, tiny granules frequently being found in cells containing large granules with none intermediate in size; and further, the staining reaction to be mentioned presently.

Since the tiny granules were not considered separately until late in the present study, data obtained regarding their specific reactions are meager. In general their reactions are similar to those of the larger forms in that they are not to be found in sections and do not stain with common histological stains. No change is noticeable in the tiny granules after they have stood in salt solution. With Romanowsky and Giemsa following fixation with osmic acid vapor, they stain solidly and but for the absence of a surrounding vacuole, are indistinguishable from the deeply staining inner body of the larger

granules. In many cases, however, the granules fail to stain at all with this technique. The similarity in staining of the tiny granules and the inner bodies of the larger ones suggests the possibility that the former are the first stages in the formation of the larger granules. If such were the case, presumably these would become surrounded with calcium carbonate or other inorganic material and thus give rise directly to the larger spherical, refractive forms. Direct division of the tiny granules has not been observed, though the occurrence of elongate forms and pairs suggests that this may occur.

3. In a number of specimens taken from a hive in winter quarters, there were observed in addition to the refractive granules, irregular, slightly refractive bodies, measuring 1 to 1.5μ in diameter. While in such specimens these occurred in almost every epithelial cell, only rarely was there more than one in each cell. These irregular bodies could not be distinguished in stained cover-glass preparations or in sections. On account of the limited material further data were not obtained.

4. Toward the close of this study there was noted for the first time in bees taken during the spring from blossoms and from hives of different apiaries, the presence of slender rods in epithelial cells of the ventriculus (Figs. 21-23). These rods measured 0.1 to 0.2μ in diameter by 1 to 2μ in length. They were colorless and not refractive and could be distinguished from the cytaplasm in which they were suspended only with some difficulty. In this respect they resembled somewhat certain spirochaetes. The number to be found in any cell varied greatly. Many contained but very few, in which cases it was easy to observe the individual rods. These frequently exhibited slight Brownian movement. Other cells contained many rods which formed compact masses, usually at the periphery (Fig. 21). In such masses it was difficult to resolve the individuals. Many cells lacked these bodies, though they could be found in nearly all bees after prolonged search. In addition to the rods the refractive granules were present in apparently normal manner. Repeated efforts to stain the rods with Romanowsky, Giemsa and hematoxylin failed completely, not the slightest trace of them being found after staining, either in cover-glass preparations or in sections. The possibility that these bodies are needle-like crystals is not precluded.

5. Occasionally there are noted one to many spherical vacuoles in epithelial cells. They appear to consist of a liquid which is colorless or at times shows traces of pink. When present there are usually one to several vacuoles, about one-fifth the diameter of the cell. Rarely the cell is completely filled with small vacuoles. These are not to be confused with those surrounding the planonts of *Nosema apis* since no bodies can be made out within the vacuoles, either in fresh or

stained preparations. Since vacuoles have been observed more frequently and in greater numbers in bees from colonies suffering from various disorders, than in healthy bees, it is possible that the vacuoles indicate a pathological condition.

From the scope of the present study, the foregoing survey of the histology and cytology of the honey-bee's ventriculus has necessarily been in some respects a catalogue, rather than a discussion, of the many structures concerned. Their consideration has necessarily been almost entirely from the morphological point of view, and even in this respect much remains yet to be done, while the many related physiological problems, the solution of which is vital to an understanding of the whole subject, remain a practically unexplored field.

THE RELATION OF CYTOPLASMIC INCLUSIONS TO INTRACELLULAR MICRO-ORGANISMS

It is seen from the foregoing that in the cytoplasm of the epithelial cells are found a number of different bodies, of the nature, origin, behavior and ultimate fate of which it is not possible at this time to give a satisfactory explanation. A cursory examination of insect tissues in general reveals the fact that granules or other inclusions are characteristic not alone of the epithelial cells of the honey-bee's midintestine, but indeed of nearly every tissue of every insect. The same is true for many other arthropods. The further study of these inclusions becomes of immediate importance when the common occurrence in insects of intracellular parasites and symbionts is considered.

Intracellular parasitism in insects is of such common occurrence as to constitute an almost universal phenomenon. Many such parasites possess complicated life cycles and our knowledge of many is but fragmentary. Furthermore, in a number of cases in which organisms pathogenic for vertebrates pass a portion of their life cycle in the tissues of insects or arachnids, no trace of the organism has been found in the arthropod host. It is conceivable that the forms assumed by the organism in the arthropod tissues so closely resemble granules or other normal inclusions, and are thus so thoroughly masked, that they easily escape detection. That this may well be the case is borne out by the history of Rocky Mountain spotted fever, a tick-borne disease in which the causal agent is transmitted through many generations of ticks via the egg. The organism (one related to the Rickettsia group) has only recently been discovered by Wolbach (1919). He has described three forms of the organism in the tick, namely, tiny rods in the digestive tract, and two forms of short, paired rods or cocci in various tissues, one form being intranuclear. These organisms stain only with Giemsa. In another tick-borne disease, Texas cattle fever, in which the organism is transmitted via the egg to the second gen-

eration of ticks, the form present in the tick has not been described, although the form in the vertebrate host is well known. In certain tick-borne spirochaetoses there have been described or suspected granule stages of the organism in the tick (Leishman 1910, African relapsing fever; Balfour 1911, 1912, Hindle 1911, spirochaetoses of fowls. Marchoux and Couvy (1913) dissented from Hindle's view, claiming the granules found in ticks to be normal and not derived from spirochaetes.) The literature of Rickettsia in connection with typhus and trench fever, transmitted by lice, is suggestive. These organisms were held by several investigators to be merely normal granules (Wolbach, Todd and Palfrey 1922, bibliography). Tiny granule-like organisms of the Rickettsia type, both intra- and extra-cellular, some transmitted hereditarily, have been found in more than a dozen insects and ticks, and are apparently of general distribution throughout these groups. In his work on the etiology of yellow fever, Noguchi (1919) discovered that in one culture of the causal organism, *Leptospira icteroides,* the typical spiral form had disappeared, while in its place were many tiny granules. The spiral form was later recovered in transfers from this culture. Noguchi suggested that the organism may possess a granule stage in its life history—an especially interesting possibility in view of the fact that the disease is carried by an insect and the further fact that the form present in the insect has not yet been described. The few examples cited will serve to show that in many instances organisms in the tissues of insects can be recognized as such only with difficulty or not at all, and that as a result the many cell inclusions must be studied carefully and in some cases are rightfully to be placed under suspicion.

The problem is further complicated by the common occurrence of intracellular symbionts, certain organisms being constantly present in definite regions or organs or cells of various species of insects. These organisms are transmitted from generation to generation through the egg, and their development in the embryo and adult follows a course as definite as that of any organ of the insect itself (Buchner 1912, 1921, bibliography). Since in the species concerned there are no uninfected individuals for comparison, many symbionts now well known and easily recognized, were for some time believed to be merely cell inclusions. This view, for example, was held concerning the large, rod-shaped bacteroids of the Blattidae. The extent to which intracellular organisms have been overlooked and misinterpreted, and the probability that many symbionts or parasites of the granule-like Rickettsia type are yet to be described, make necessary great caution in passing judgment on the cytoplasmic inclusions of insect tissues.

Koehler (1920) considered the possibility of the refractive granules being symbionts, but abandoned the idea on finding them, as she

believed, to be composed almost entirely of calcium carbonate. There may be noted, however, a number of cases in which symbionts are found in the epithelium of the mid-intestine of other insects, notably the bacteroids of the carpenter ant, Camponotus, and yeast-like organisms in the "drug-store" beetle, *Sitodrepa panicea* (Buchner 1912), the pith-eating Lepidopterous larva, *Nonagria typhae* (Portier 1911), and certain of the pupiparous Diptera such as Glossina and Melophagus (Roubaud 1919). There has not been described any organism living in symbiosis with the honey-bee.

THE VENTRICULUS INFECTED WITH *NOSEMA APIS*

In this study of *Nosema apis* the chief purpose has been the determination of changes produced by the parasite in the host tissues, rather than a study of the parasite itself. A consideration of the latter, however, is a necessary prelude to a study of the pathological histology of the host. In the literature of the Microsporidian parasites found in honey-bees throughout the world, the organisms have in nearly all cases been identified as *Nosema apis* Zander. As noted below, there is strong probability that at least one other Microsporidian species may be present. Identification at best is somewhat uncertain because of the technical difficulties in determining accurately all the different stages in the life cycle, and further because of the conflicting accounts of various writers. The parasite most commonly found in the writer's material has been provisionally identified as *Nosema apis*.

The morphology and life-history of the parasite have been discussed in some detail by Zander (1911, 1921) and by Fantham and Porter (1912a, 1912b), while White (1919) has given much information concerning the resistance of the spores to chemical and physical agents. Kudo (1921) has added some notes concerning the morphology of the spore of *Nosema apis* and has recorded the occasional occurrence of an undetermined Microsporidian, possibly a new species of Nosema, in the ventriculus of the honey-bee.

The morphology and development of *Nosema apis* as described by Zander and Fantham and Porter, which is very similar to that given by Stempell (1909) for *Nosema bombycis* Nägeli, is reviewed below, together with observations of the writer.

The spore of *Nosema apis* is swallowed by the bee with food or water and enters the mid-intestine. Here the spore germinates, this process consisting of the discharge of the coiled polar filament through a pore at one end of the spore, followed by the issuance of the ameboid germ. According to Fantham and Porter this body contains two nuclei which appear as refractive spots. Each amebula gives rise to one or sometimes two uninucleate bodies, termed planonts, which move slowly about by means of pseudopodia. These may multiply in the

lumen of the intestine, giving rise to colonies of young planonts, "each of which moves about over the epithelial surface of the gut, and finally penetrates between the cells or directly enters them and becomes intracellular. . . . The method of penetration of a cell by a planont is most difficult of observation, though it has been seen in life on a few occasions." Unfortunately details of the actual process of penetration were not given. Neither Zander nor Fantham and Porter have taken into consideration the peritrophic membranes or the striated border in considering the movements of the planonts from the germinating spore to the penetration of the epithelial cells. Presumably the spores after they enter the intestine are contained within the innermost peritrophic membrane and are thus separated from the epithelium by several layers of these membranes. The latter are for the most part effective barriers to bacteria and food materials, and the method by which the planont penetrates them is not clear. Fantham and Porter stated that the planonts free in the lumen "stain fairly well with Romanowsky stains" but only moderately after they have become intracellular, and are distinguishable from the cell contents only with some difficulty. These investigators were able to distinguish planonts from other organisms such as yeasts "(1) by their movements, (2) the stainability of the nucleus, (3) by chemical tests, of which that for fungus cellulose is the chief. Planonts have no fungus cellulose." In addition to penetrating epithelial cells directly from the lumen, the planonts "may reach the haemocoel or body cavity of the bee and remain there in a resting condition for some time. They lose their motility temporarily, become rounded or oval and lie quiescent. After an interval their activity returns and from the haemocoel they retreat between the cells to the epithelium of the gut, which they gradually penetrate." These investigators found planonts and meronts in the blood of the bee, but their evidence for the return of the planont from the haemocoel to the epithelium, involving a second penetration of a well developed, chitinous basement membrane, was not indicated. White (1919) stated that "in infecting the stomach the parasite reaches the basement membrane but does not penetrate it."

Arrived within an epithelial cell "the active motile planont becomes passive, loses its pseudopodia and enters on a growing stage," which is followed by multiplication after a short time (Fantham and Porter). The parasite at this stage is known as a meront. The round or oval, uninucleate meront increases in size and divides most commonly by binary fission, though there may be produced chains of daughter meronts or large multinucleate meronts which later divide into typical meronts. In Zander's (1921) diagram and microphotographs, the meront stage is represented by "nests" of greatly elongate forms or

chains of as many as eleven individuals, the isolated oval forms being greatly in the minority. Elongate forms were not common and chains were not observed at all in the writer's material.

In distinguishing the planonts and meronts from normal cell inclusions the appearance of the parasites in the fresh condition is a particularly important consideration. Fantham and Porter stated that the planonts after they have become intracellular are very difficult to see either fresh or stained. Stempell (1909) found the same to be true of the planonts of *Nosema bombycis*. In the writer's experience the intracellular planont stage has never been made out with absolute certainty. Apparently this form is similar in density, refractivity, etc., to the protoplasm of the cell and stains with the same intensity. Fantham and Porter have not described the appearance of the living meront, though in their figures drawn from fresh preparations, meronts are shown as rounded or oval bodies, all with distinct nuclei and with finely granular, or in some cases alveolar, cytoplasm. In the writer's preparations the meronts were mostly spherical or oval, and greatly resembled vacuoles with a slightly refractive outer portion. The protoplasm of the meront was clear, homogeneous and apparently of about the same density as the cytoplasm of the host cell (Figs. 24-26, 33-38). Only very rarely could structures be seen within the meronts, these being interpreted as nuclei of the latter (Figs. 26, 32). Stempell (1909) reported that the meronts of both *Nosema bombycis* and *Thelohania mülleri* possess a pellicle-like outer layer of protoplasm. It is perhaps such a structure which appears as the slightly refractive covering of meronts of *Nosema apis*. The meronts are usually somewhat larger than the mature spores. Greatly enlarged multinucleate meronts as described and figured by Fantham and Porter have not been observed by the writer. Their Figure 47, evidently drawn from a fresh preparation, shows portions of four host cells containing five large multinucleate and two uninucleate meronts. In addition there are oval or elongate bodies of about the size of mature spores, which apparently represent the host nuclei. From the writer's observations the nuclei of the epithelial cells are almost always very much larger than Nosema spores, even the small nuclei of the regenerative cells being usually at least twice as large as the spores. The nuclei frequently appear as large spheres with a number of distinct spherical or somewhat irregular bodies suspended within (Fig. 3), closely resembling the large multinucleate meronts shown by Fantham and Porter. There might, as a result, be a possibility in the examination of fresh material, of mistaking a large meront for the host nucleus, and an abnormally small nucleus for a parasite, and vice versa.

There was occasionally encountered another form of meront which may not be that of *Nosema apis* but may be one stage of the undeter-

mined Microsporidian found by Kudo (1921). These forms were elongate bodies, some of which were constricted at the middle and nearly all of which were bent forming an obtuse angle (Figs. 39-48). The elongate, bent forms were usually highly refractive, resembling in this respect, the mature spore. No internal structure could be made out. The forms undergoing division were somewhat less refractive and usually contained at the constriction a distinct vacuole, which seemed also to be undergoing division (Fig. 48). Shorter forms with a vacuole at one end also occurred, together with short refractive forms without a vacuole, resembling the ordinary mature spore of *Nosema apis*. Whether these are spores or whether they are meronts which later become the refractive elongate and dividing forms with vacuoles, could not be determined from the limited material. Accompanying the forms just described were occasionally numbers of mature spores which were refractive and contained a large vacuole at one end, similar in this respect to the spores of Kudo's Microsporidian. That these forms, both meronts and spores, are distinct from *Nosema apis* is indicated by a characteristic staining reaction. With the Romanowsky stain used by the writer, the meronts and spores of *Nosema apis* stain blue with rarely any trace of red. On the other hand, the vacuolate forms contain a more or less irregular group of ruby-red granules within the vacuole. Infection by this form was almost always accompanied by infection with *Nosema apis,* small scattered areas being occupied by the vacuolate parasites. In one series of sections, for example, the epithelial cells were all heavily infected with *Nosema apis,* while one Malpighian tube contained exclusively forms with the brilliant red granules.

Following multiplication of the meronts within the cell, spore formation takes place. This process has been described in some detail by Fantham and Porter. Each ultimate meront undergoes a number of somewhat complex cytoplasmic and nuclear changes, resulting in the formation of a single spore with several nuclei and a polar capsule containing a long coiled thread, the polar filament, the whole being provided with a dense, refractive spore wall. Fantham and Porter stated that "when young the contents of the spores are finely granular and a single nucleus can be seen within them in life." Such forms have not been seen in the writer's preparations. The protoplasm of young spores is denser than that of meronts and appears homogeneous throughout except for the frequent occurrence of a vacuole at one or both ends as described by Fantham and Porter. At this stage there are discernible at times several cross striations in the protoplasm of both fresh and stained spores. According to both Zander and Fantham and Porter, these may represent the polar filament in process of formation. The vacuoles and cross striations are more marked after

the spores have remained some time in salt solution. In older and mature spores, the formation of the refractive spore wall makes examination of structural details impossible in the fresh condition. Fantham and Porter's detailed description of the structure of the mature spore differs in many important respects from that of Kudo (1921). The former represent the spore as containing a binucleate, girdle-like sporoplasm at the equator of the spore, surrounding the polar capsule with its coiled polar filament. There are in addition three accessory nuclei. Kudo's diagram shows a uninucleate, rounded sporoplasm at one end of the spore, the polar capsule being at the other end, with no accessory nuclei. It may be mentioned that with ordinary histological technique these details cannot be made out at all. The spores stain as shown in Figure 4, there being a clear space at one end, traversed by an axial thread, probably the base of the polar filament. The remainder of the spore contents, including probably the polar filament, forms a deeply staining, elongate triangular mass.

Spores contained in epithelial cells which are discharged into the lumen of the intestine, are eventually voided with the feces and may then serve to infect bees taking food or water thus contaminated. To what extent reinfection by parasites produced within the same ventriculus may take place, has not been determined. Fantham and Porter (1912b) believed this to be possible to a limited extent by means of mature spores. Zander (1921) believed that reinfection may take place by means of younger stages as well as spores discharged into the lumen. Maassen (1912) held that reinfection by means of mature spores was impossible, though it could probably be brought about by younger forms. Zander held that only by some method of reinfection could be explained the fact that frequently the entire epithelial layer is filled with parasites. This view is supported by a number of facts. It has been the writer's experience that in the summer and early fall the number of infected bees is small. About October or November it is common to find one infected bee in every twenty or thirty, and it is noteworthy that almost without exception such infected bees are very heavily infected, while no traces of parasites can be found in other bees from the same hives. Equal opportunity for ingestion of spores may be assumed for all bees of the same hive. The heavy infection of a few out of many uninfected individuals may be due to differential susceptibility, or to the spread of the parasites within the host following initial infection by relatively few spores. The latter would seem the more probable, and is borne out by the occurrence of areas parasitized exclusively by the undetermined Microsporidian, surrounded by areas containing only *Nosema apis*.

All accounts agree that infection with Nosema is most frequently found in workers, though drones and queens are susceptible. How-

ever, the drones and queens of even heavily infected colonies may escape infection altogether. As to the larvae, reports differ. White (1919) as a result of inoculation experiments concluded that the larvae are not susceptible. Fantham and Porter (1912b), however, reported finding meronts and occasionally spores in cells of the larval mid-intestine, and Maassen (1919) reported infection of the brood. The infection is usually limited to epithelial cells of the ventriculus, whether of workers, drones or queens, though in heavily infected specimens it is not uncommon to find the parasites within cells of the basal portion of the Malpighian tubes. Fantham and Porter (1912b) have reported the presence of planonts and meronts in the body fluid. Parasites have not been found in cells of the esophageal valve nor in those of the small intestine.

CHANGES IN THE VENTRICULUS PRODUCED BY NOSEMA

The presence of the parasites produces certain changes in the ventriculus which are recognizable with the naked eye. When the cells of the epthelium are filled with spores, the translucent, reddish brown appearance becomes a milky or chalky whiteness. On being crushed under a cover-glass the entire ventriculus may disintegrate forming a milky mass. White (1919) found that "the organ is often increased in size, the circular constrictions are less marked, and the transparency is diminished. In late stages of the disease, however, the stomach approaches the normal in size and the constrictions are again well marked." Swollen forms were not common in the writer's material. It is only in advanced stages of infection, however, that the presence of the parasites can be detected by the milky or chalky appearance, and not always with certainty even then. While normally reddish brown in color, the ventriculus of healthy bees varies from a whitish yellow to dark brown. When the epithelial cells of the lighter colored specimens are filled with large refractive granules, the outward appearance is somewhat similar to that of an infected ventriculus. Microscopic examination is the only certain method of determining the presence or absence of the parasites. The consistency of the ventriculus is markedly different in advanced stages of infection. Normally it is firm and when crushed under a cover-glass tends to recover its form. The peritrophic membranes remain together in a mass. The heavily infected ventriculus has lost its firmness and elasticity and disintegrates readily. Many cells filled with spores remain intact, but an enormous number of spores escape from the easily ruptured cells. Peritrophic membranes are rarely recognizable as such.

Changes in the general shape and arrangement of epithelial cells and other structures due to the presence of Nosema become apparent

only after the organisms have been fairly well distributed throughout the intestine and the majority of cells have become infected. Newly infected cells, except for certain cytoplasmic features, appear to be quite similar to their uninfected neighbors (Fig. 3). Infection may proceed to a stage where the majority of the cells contain a number of meronts, or even spores, before any differences are noted other than the presence of the parasites themselves. This is true of fresh as well as stained tissues. The size and shape of the cell and the appearance of the cytoplasm are unchanged. The striated border and peritrophic membranes are normal. The formation and discharge of secretion cells proceeds as usual. The small, stalked secretion cells which have been budded off from the larger epithelial cells may or may not contain meronts or spores.

After the epitelium has been completely parasitized and the infection has persisted some little time, a number of marked changes are recognizable. The musculature and basement membrane alone are unaffected. The epithelial cells are in general larger, evidenced by their greater length in sections, and increased diameter as seen in fresh preparations. Fantham and Porter (1912b) stated that "the passage of the spores outwards into the lumen of the gut causes tears and gaps to appear in the intestinal wall," such injured cells being replaced by new ones. Liberation of spores in this fashion has not been observed by the writer, the parasites practically always being within host cells until the latter have left the epithelium and have disintegrated in the lumen. These writers further stated that "when an intense infection is present, the bee seems to lose its power of reforming cells." Quite the reverse would seem to be true from the writer's experience. There is a marked tendency toward increased proliferation of cells in heavily infected individuals, resulting in an abnormally thickened epithelium, composed of greatly elongate cells attached to the basement membrane and occasionally there are elongate secretion cells arising from these (Fig. 7). This condition may also be explained by delay in the discharge of secretion cells into the lumen. Excessive proliferation and elongation of cells are by no means invariable accompaniments of heavy infection, nor are they found only in infected tissues. In many infected areas the cells are not at all elongate. Figures 6 and 7 represent different regions of the same section, the cells of one being enormously elongate, while those of the other are quite the reverse, the degree of infection being the same in both. One method of formation of secretion cells is strikingly shown in Figure 7. Small cap-like cells are seen covering the ends of several epithelial cells, the former being entirely free of parasites, while the parent cells are filled with them. This method of secretion-cell

formation, as already described, has also been observed in uninfected specimens, which indicates that even heavily infected cells may function normally in some respects.

In heavily infected areas Fantham and Porter (1912b) found "the secretory epithelium reduced to the condition of a pulp or sponge-like meshwork, enclosing large colonies of meronts and spores within its strands." Degeneration of the cells to this extent has not been noted in the writer's material. With the exception of the region next the basement membrane, the cell outlines are usually clear (Figs. 4-7). The nidi, the cells of which may contain few or no parasites, even in heavily infected individuals, may be little changed or, on the other hand, may not be recognizable at all, as shown in Figures 6 and 7, drawn from different areas of the same section.

With advance in the degree of infection, the striated border becomes less marked. In fresh mounts the epithelial cells filled with spores usually lack the radiating hair-like processes so conspicuous in the case of healthy bees. In sections the hair-like structure may be quite definite in some areas, in others there may be merely a granular mass (Figs. 5-7). Along with the striated border, the peritrophic membranes become more indefinite and their formation more uncertain as infection progresses. Frequently the peritrophic membranes are scarcely recognizable, the lumen of the ventriculus being entirely filled with spores, either free or within discharged cells. In such sections the space between the spores may be occupied by a finely granular substance, together with bacteria and yeasts. Maassen (1919) noted the occurrence of greater numbers of bacteria in infected than in uninfected individuals.

The morphologically recognizable effects of Nosema upon the contents of the epithelial cells are few. The host nuclei appear not to be affected, since in heavily infected cells they are not appreciably different from those of normal cells. Parasites within host nuclei have not been mentioned by the various investigators, nor has this condition been noted by the writer. This point, however, is difficult of determination with certainty, for in many heavily infected cells there are no nuclei. It may be that in such cases the nuclei have been destroyed, or on the other hand, the cells may be secretion cells which never possessed nuclei.

Fantham and Porter (1912b) found a clear space or "halo" surrounding meronts a short time after they had become intracellular, this halo becoming more marked with the growth of the meront. These writers considered the clear space to be possibly an alteration of the concentration of the liquid surrounding the parasite, due perhaps to the protoplasm having been digested by the parasite, or "to the removal by simple absorption from the cytoplasm of the invaded

cell of various granular constituents, used by the parasite as food." It is not clear whether the "granular constituents" refer to the conspicuous cell inclusions, or to the protoplasm itself which is at times indistinctly finely granular. Clear spaces or vacuoles surrounding the meronts have been observed but rarely by the writer in fresh material, though in sections a rather definite space may be found surrounding each parasite (Fig. 4). Both Zander (1921) and Fantham and Porter (1912b) have figured "nests" of meronts lying within large clear spaces.

The effect of *Nosema apis* on the various cell inclusions has received little attention. Fantham and Porter have not mentioned these bodies specifically, nor do the latter appear in their figures drawn from fresh preparations, though large numbers of the refractive, spherical granules appear in certain of their microphotographs (1912a). Zander (1921) gave a figure of a healthy cell filled with "Kalkkörperchen" or refractive granules, and showed a few of these in a cell filled with spores. Koehler (1920), who was led to the investigation of the epithelial inclusions in connection with a study of Nosema-infected bees, stated that in cells filled with spores the "calcium-granules" are few or have disappeared altogether. She suggested that the pathological effect of the parasite, while it might be the effect of toxins, might also be due to a disturbance of digestion and resorption resulting from a deficiency in calcium. On testing for calcium in the spore walls, Koehler obtained only negative results, so that apparently the calcium of the granules is not used directly by the parasites. However, she considered the disturbance of the calcium cycle a probability.

The writer's observations agree with those of Koehler as to the decrease in number of the refractive granules in heavily infected cells. It is only rarely, however, that the larger, nucleate cells lack these entirely, though the smaller secretion cells are often without granules. There is no change in appearance or staining reaction of these granules, though a few abnormal forms have been noted (Fig. 31). There is, however, in nearly all cases a very marked increase in the number of the very tiny granules described above (p. 123). These are spherical, slightly irregular or rod-shaped, and very frequently in pairs. In infected specimens it often happens that such tiny granules predominate even in cells which contain no parasites. With Romanowsky these tiny bodies may stain deeply, though quite as frequently they are not to be found after staining, or else their numbers are greatly reduced. Whether these tiny granules represent the central portion of the larger, refractive granules minus their inorganic outer portion, and are thus a degeneration stage of the latter, cannot be stated. It would seem certain, however, that their marked increase

in number is due in some way to the presence of the parasites. The appearance of the cytoplasm is unchanged, though it is, of course, ultimately replaced in large measure by the parasites. The slender, non-refractive rods (p. 124) have been observed in the epithelial cells of a few heavily infected individuals.

SUMMARY OF PATHOLOGICAL CONDITIONS ASSOCIATED WITH NOSEMA

From the foregoing it is seen that throughout infection the epithelial cells retain their identity, the cells not being "destroyed" at all, strictly speaking, since they disintegrate only after they have reached the lumen. Though the cytoplasm is largely replaced by parasites, the nuclei and cell membranes seem uninjured, though the latter are probably more easily ruptured. Along with destruction of the cytoplasm, changes in relative numbers of various cell inclusions take place. There appears a tendency toward increased proliferation of epithelial cells, with consequent thickening of the epithelium. With the advance in infection, the formation of striated border and peritrophic membrane becomes seriously deranged. Changes in the appearance and contents of the organ harboring the parasites occur only with heavy infection. The color changes from red or brown to chalky white, and the firmness and elasticity of the tissues are lost.

From the behavior of infected bees it is seen that these pathological conditions do not immediately produce outward symptoms of disease. In a colony known to harbor the parasites, it is impossible to distinguish from appearance or behavior, the infected from the uninfected individuals, except those actually dying of the disease. Since in this latter condition the usual symptoms, i. e., crawling, inability to fly, distended abdomen, etc., are quite as characteristic of disorders not associated with Nosema, microscopic examination is the only certain method of diagnosis. Since it is thus possible for the infection to be in an advanced stage without having any apparent effect on the behavior of the bee, the ultimate pathological effect, namely, the weakening and death of the bee, would appear to be due, not to any one of the pathological conditions enumerated above, but to the collective and cumulative effect of some or all of them. Toxins produced by the parasite, if any, would seem to make little or no contribution to the pathological condition, since their effects could be expected to manifest themselves during the growing stages of the parasite. Until more is known of the physiology of the honey-bee and of insects in general, the most plausible explanation of the condition, and the one commonly advanced, is that some derangement of the digestive processes takes place, which leads to the malnutrition and hence the weakening and ultimate death of the host.

A PATHOLOGICAL CONDITION OF THE VENTRICULUS NOT ASSOCIATED WITH NOSEMA

In June 1920 there came to the notice of the writer a hive which had been suffering a marked and constant loss of bees since it was set out in the spring. There were generally to be found on the ground near the hive as many as a hundred or more bees which were in great distress, crawling about excitedly or sluggishly, or lying motionless except for occasional trembling movements of wings and legs. Examination failed to reveal the presence of Nosema and there was no apparent cause for the death of the bees in such numbers. The colony was an isolated one, located in the residential district of St. Anthony Park, St. Paul, about one-quarter of a mile from the apiary at University Farm. No marked loss of bees was noted in the latter apiary.

The mid-intestines of the diseased bees examined in June 1920 were mostly pale yellowish, and decidedly smaller in size than normal. The contents were colorless or pale brown. The hind-intestine had a pale, watery appearance. When the ventriculus was crushed under a cover-glass, the epithelial cells which became detached lacked the elasticity of normal cells. They tended to retain the elongate-pyriform shape instead of becoming spherical when the pressure of adjoining cells was released. Apparently normal granules were present in the cells, though in some specimens there were in addition refractive spheres or globules, possibly of some liquid, very much larger than the ordinary refractive granules. Sections revealed a rather striking pathological condition, totally unlike that encountered in Nosema-infected bees. The entire cellular structure had in many regions become a coarsely granular mass. The outlines of many cells and nuclei were wholly indefinite (Fig. 8). In certain regions there appeared to have taken place an excessive and irregular proliferation of cells, the mass of discharged cells forming a layer thicker than the epithelium itself. Parts of the epithelium seemed to have been shed into the lumen *en masse,* the more or less intact epithelial layer being separated from the basement membrane by a considerable area filled with coarse, deeply staining granules. In certain cases, as shown in Figure 8, the epithelium had been given off in a body, but a new epithelium had been formed beneath the old. As a result there were two layers of epithelium, both showing the degenerate, granular condition. The inner layer was broken or had completely degenerated in several places, but portions of the striated border were present together with a peritrophic membrane in process of formation, and several of the latter in the lumen completely formed. On the surface of the layer resting on the basement membrane, was an unbroken striated border with the beginning of a peritrophic membrane. The basal por-

tion of this epithelial layer had lost all trace of cell outlines, being a uniformly granular area. The Malpighian tubes and muscle fibers surrounding the ventriculus were also degenerate.

Further examination of bees from this colony was not made in the season of 1920, though the owner stated that the loss of bees continued to a certain extent throughout the entire summer. The colony yielded little or no surplus honey, but was able to winter over. Shortly after it was set out in the spring of 1921 a similar loss of bees was noted. The number of stricken bees to be found at any one time, however, was rarely over forty or fifty and at times not more than a dozen. On examination the ventriculus was found to be pale and translucent, with a dark mass at the posterior end formed by the contents. The ventriculus was usually of less than normal size and was frequently of uneven diameter. Within the epithelial cells were found large numbers of the non-refractive, slender rods described above (p. 124). Since these rods were also found in healthy bees from other apiaries, no pathological significance was attached to them. In addition there were noted the abnormally large refractive bodies found the previous summer, and also a number of vacuoles. One apparently healthy bee, taken from the entrance, was heavily infected with *Nosema apis*. This parasite was not found in any other individuals, whether active or crawling. In the few specimens sectioned, the striking pathological condition found the previous season was not apparent. The only abnormal structures noted were occasional large spheres, staining lightly but uniformly with eosin. The loss of bees continued until shortly after July 1, 1921, about which time the colony was requeened.

The cause of this pathological condition, and whether or not it is infectious, are not known. It is merely one more example of the many disorders of the adult honey-bee which cannot be distinguished from each other with certainty until our knowledge of insect physiology, and of the normal and pathological histology, not alone of the mid-intestine, but of all other organs as well, is greatly extended.

The writer gratefully acknowledges the aid and encouragement of Professor C. W. Howard, under whom the work was begun, and of Dr. William A. Riley, under whose direction it was continued.

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Explanation of Figures

Figures 1 to 9 drawn with aid of camera lucida from stained preparations; Figures 10 and 49 drawn with aid of camera lucida from fresh material.

Explanation of Plate IX

1. Normal ventriculus, longi-section. Gilson. x 350.

2. Dividing nucleus of regeneration cell. Gilson. x 790.

3. Cross-section of epithelium, one cell containing meronts of *Nosema apis*. Vacuoles in cytoplasm represent refractive granules of fresh tissue. Osmic acid vapor. x 790.

4. Cells containing meronts and spores surrounded by clear areas in cytoplasm. Gilson. x 770.

5. Longi-section, junction of heavily infected ventriculus with small intestine; base of Malpighian tube slightly infected. Infected area ends abruptly with beginning of small intestine. Gilson. x 180.

6. Cross-section, heavily infected ventriculus; shape and arrangement of cells nearly normal. (Compare with Figure 7). Sublimate-alcohol. x 180.

7. Cross-section, same specimen as Figure 6. Epithelium enormously thickened as result of elongation and increase in number of cells; several cap-like secretion cells, some with cross-walls; nidi not recognizable as such. x 180.

8. Cross-section, ventriculus; pathological condition not associated with *Nosema*. Two epithelial layers apparently due to sloughing and regeneration: sloughed portion, *A*, nearly intact with striated border and peritrophic membrane; newer epithelial layer, *B*, resting on basement membrane, degenerate but with striated border and peritrophic membrane forming. Malpighian tube, *m. t.*, and muscle fibers, *m. f.*, degenerate. Hollande. x 325.

9. Refractive granules from epithelial cell, cover-glass preparation, osmic acid vapor, Romanowsky stain. Granules appear as vacuoles embedded in blue cytoplasm, each with blue-purple inner body. x 1120.

PLATE IX

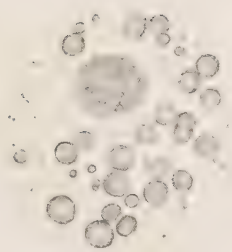
EXPLANATION OF PLATE X

10. Isolated epithelial cell with striated border. The cytoplasm contains large, spherical, refractive granules and tiny, somewhat irregular granules. x. 1910.

11-18. Secretion cells. x 1910.

19, 20. Stalked secretion cells with striated border. x 1910.

21-23 Epithelial cells containing slender, non-refractive rods in addition to normal, refractive granules. Figures 21 and 22, x 1910; Figure 23, x 1810.



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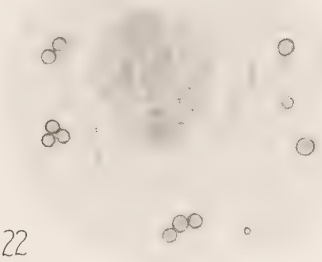

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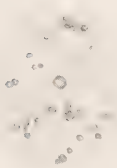

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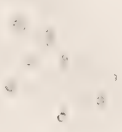

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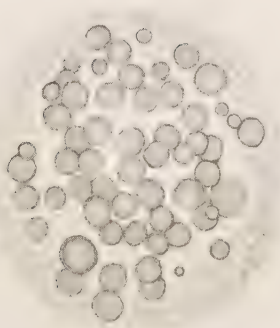

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PLATE X

EXPLANATION OF PLATE XI

24, 25. Epithelial cells containing meronts of *Nosema apis* and many tiny, irregular granules. x 1810.

26. Epithelial cells containing spores and meronts, the latter resembling vacuoles. The cytoplasm contains spherical, refractive granules and tiny, irregular granules. x 1810.

27. Double, refractive granule from epithelial cell, suggesting a division form of the spherical, refractive granules. x 1810.

28-30. Refractive granules after standing in Locke's solution one to two hours. x 1810.

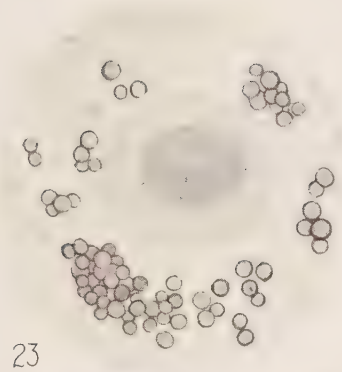
31. Abnormal forms of refractive granules from heavily infected epithelial cells. x 1810.

32-38. Young meronts of either *Nosema apis* or undetermined Microsporidian. x 1810.

39-48. Meronts, probably of undetermined Microsporidian. x 1810.

49. Young spore, *Nosema apis*. x 1810.

50. Epithelial cell filled with spores, together with a number of spherical, refractive granules. Microphotograph, fresh preparation. x 890.



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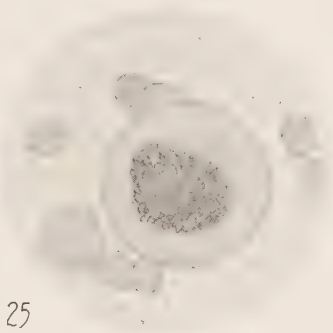

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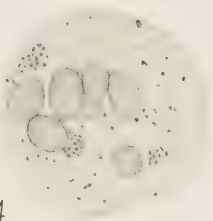

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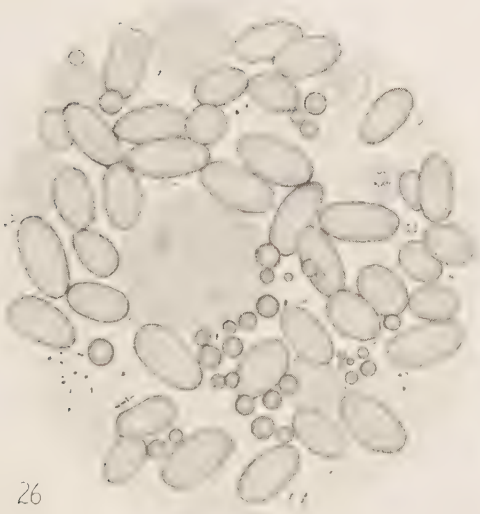

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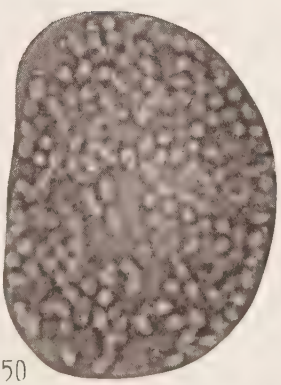

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PLATE XI

OBSERVATIONS ON THE MORPHOLOGY AND LIFE CYCLE OF *CRITHIDIA GERRIDIS* PATTON IN THE WATER-STRIDER, *GERRIS REMIGIS* SAY*

ELERY R. BECKER

This paper is a contribution to the morphology and life cycle of *Crithidia gerridis* Patton in water-striders. Two investigators, Patton and Porter, have already published reports on this subject. Patton (1908) while working in India was the first to describe flagellates from the Heteropteran bugs, *Gerris fossarum, Microvelia* sp., and *Perittopus* sp. Porter (1909) studied the morphology and life history of a similar flagellate found in the British species, *Gerris paludum.* Fantham recently reported the presence of a crithidia in the water-striders of South Africa. The writer has recently examined various species of North American water-striders, and has found instances of infection of the digestive tract by flagellates belonging to the genus Crithidia in the following species: *Gerris remigis, Gerris marginatus, Gerris rufoscutellatus,* and *Microvelia american.*†

Minchin and Thompson (1915) found that the various stages of *Trypanosoma lewisi* in the rat flea bore a definite relationship to the various parts of the intestine, and were able to follow chronologically the development in the intestine of the invertebrate host. McCulloch (1919) found that the life cycle of *Crithidia euryophthalmi,* parasitic in *Euryophthalmus convivus,* "can be correlated advantageously with the life history of *T. lewisi* in the invertebrate host, the flea." In my investigation of *Crithidia gerridis* an attempt has been made to learn how closely its life history corresponds to that of the above mentioned flagellates. The host *Gerris remigis* was selected for use because it was the most easily obtainable in large numbers.

An average specimen of *Gerris remigis,* measuring about 1.5 cm. from the vertex of the head to the tip of the abdomen, has a digestive tract from 2.5 to 3.0 cm. in length. Its general gross structure is represented in Figure 1. The esophagus is a thin-walled narrow tube, joining the first stomach with its wall of tall glandular epithelial cells. The long, narrow second stomach is much folded, and may present certain temporary enlargements and constrictions in some specimens. Posteriorally it forms a short bell-shaped enlargement which may be designated the third stomach. This unites broadly with the ileum into

* From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

† I am indebted to Dr. H. M. Parshley for identifying these species of insects for me.

which open the four long and much entangled Malpighian tubules, two from each side. A slight constriction separates the ileum from the rectum, which when empty appears as a white thin-walled sac. No gastric ceca are present.*

TECHNIQUE EMPLOYED

The living parasites were studied in vaseline sealed cover slip preparations of the various parts of infected intestines teased up in 0.7 per cent. sodium chloride solution. Intra-vitam staining with neutral red, methylene blue, and Janus green was attempted, the last only proving valuable. Methyl green distorted the unfixed flagellates badly, but brought out very well the structure of the nucleus.

For permanent preparations I employed Heidenhain's iron-hematoxylin staining after fixation in Flemming's solution, Bouin's picro-formol, or Schaudinn's fluid heated to about 60 C. Bouin's fluid proved the most valuable fixative. The Giemsa method of staining after fixation in osmic acid vapor gave beautiful pictures, but is not so valuable for critical morphological work. Entire infected intestines were fixed in hot Schaudinn's, sectioned, and stained with Delafield's hematoxylin. For mitochondrial studies Janus green made up in the proportion of one part of Janus green to 20,000 parts of Ringer's solution was used. Permanent preparations were made by the Benda and Bensley methods.

Attempts to culture the Crithidia in Wenyon's, Novy and MacNeal, and N. N. N. media failed, probably because of inability to eliminate the growth of bacteria which is fatal to the growth of all the hemoflagellates.

PERCENTAGE OF INFECTION

An examination of a number of wild specimens of *Gerris remigis* disclosed the fact that the infection may be either extremely heavy, where the intestine is actually gorged with parasites, or so light that it is possible to demonstrate the presence of only two or three flagellates. There is always the possibility of overlooking very light infections. However, a record of the examination of two hundred water-striders collected in July, 1921, from a small pond near the fish hatchery at Cold Spring Harbor, Long Island, showed a percentage of infection of 20.5. During April, 1922, 257 water-striders were collected in the vicinity of Baltimore, and of these only six, or 2.5 per cent. were infected. The writer's experience is that the infection rate at other times of the year is about the same in this vicinity.

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* The terminology used in this description was adapted to the gut of *Gerris remigis* from that employed by Glasgow in his work on The Gastric Caeca and the Caecal Bacteria of the Heteroptera.

DISTRIBUTION IN THE HOST

The esophagus is usually free of flagellates. Except in specimens which have recently fed, the anterior end of the first stomach is also usually empty. It is in the posterior half of the first stomach where one usually finds the greatest numbers of parasites. From this point to the rectum the infection is distributed more or less unevenly.

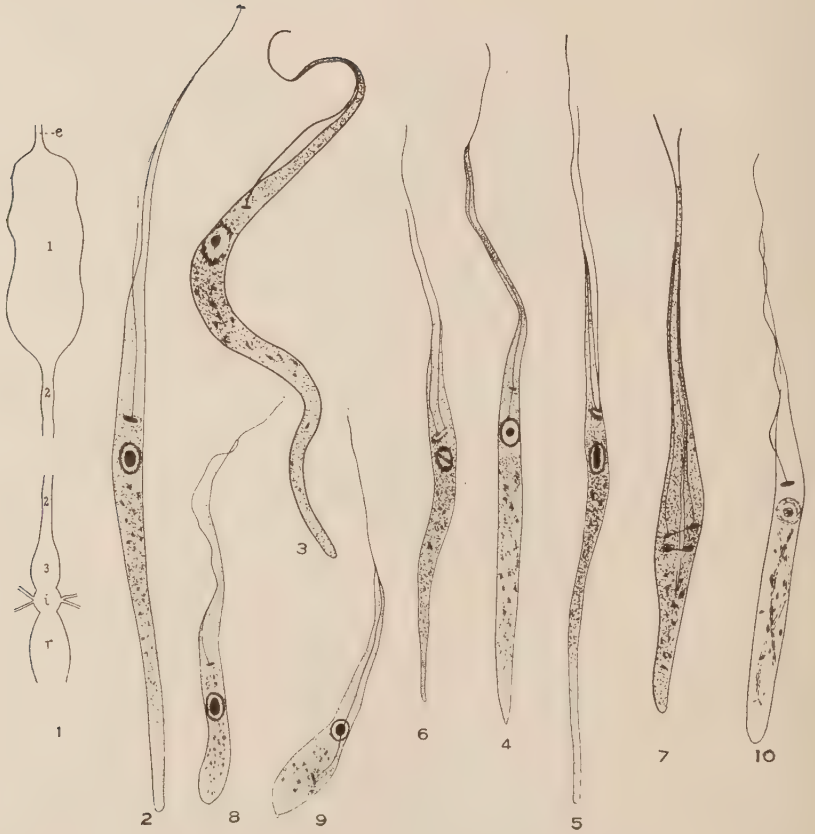
McCulloch (1919) following the terminology of Minchin and Thompson (1915) has applied the name *nectomonads* to the free-swimming flagellated forms of the Crithidia in the insect's intestine, and the name *haptomonads* to those forms which have attached themselves. In the first stomach of infected *Gerris remigis* both the free-swimming and the attached form of the parasite may be found, and I shall designate such forms as nectomonads and haptomonads respectively.

TABLE 1.—MEASUREMENTS IN MICRONS OF 100 NECTOMONADS TAKEN 10 EACH FROM 10 WATER-STRIDERS

Number of Individuals Measured	Average of Greatest Width	Average Distance from Posterior End to Nucleus	Average Distance from Nucleus to Parabasal Body	Average Distance from Parabasal Body to Anterior End	Range in Length	Average Total Length
10	2.2	5.0	1.1	16.8	18.2–25.9	22.8
10	2.2	9.8	2.1	20.8	25.0–40.4	32.8
10	1.4	19.4	2.4	21.4	33.4–54.0	43.2
10	1.8	10.5	2.2	23.2	29.9–45.5	35.9
10	1.8	9.8	1.2	18.1	20.4–39.6	29.1
10	2.5	5.9	1.3	21.1	21.3–34.5	28.3
10	2.1	8.0	1.4	22.6	22.8–40.3	32.0
10	1.9	13.6	2.2	23.9	32.2–47.1	39.6
10	1.3	19.4	2.7	23.8	40.0–60.0	45.9
10	2.5	10.4	1.9	21.4	25.5–43.6	33.9
Average	2.0	11.2	1.9	21.3	.....	34.3

Nectomonads (Fig. 2-9). Ordinarily these forms predominate in the first stomach. When the gut is ruptured they pour out in great profusion, almost emptying the gut. Some of these long, slender forms swim with great rapidity; they represent the stage of maximum activity in the life cycle of the parasite. Porter has given a good description of their movements. Others are found together in great agglomeration rosettes as described by Patton and Porter.

A series of 100 measurements showed that these nectomonads range in length from 18.2 to 60.0μ, and in width from 0.9 to 3.2μ. The average width was 2.0μ; the average distance from the posterior tip to the nucleus 11.2μ, from the nucleus to the parabasal body 1.9μ, from parabasal body to the anterior tip of flagellum 21.3μ, and the average total length was 34.3μ. I have seen only two individuals in which the parabasal body was posterior to the nucleus (Fig. 9). The foregoing table of 100 measurements of ten flagellates from each of ten insect hosts shows the amount of variation in the size and shape of the flagellate in various hosts of the same species.



Figs. 1-10.—Diagrammatic drawing of the digestive tract of *Gerris remigis*. *e*, esophagus; *1*, first stomach; *2*, second stomach, the greater part not shown; *3*, third stomach; *i*, ileum; *r*, rectum. 2. Large nectomonad, type commonly found in first and second stomachs. 3. Nectomonad with nuclear membrane composed of ring of clouded granules. 4. Nectomonad with apparent nuclear rhizoplast, undulating membrane only slightly developed. 5. Nectomonad commencing division. 6. Further division stage. 7. Parabasal body and nucleus still incompletely divided, cytoplasm commencing to split between two new individuals. 8. Nectomonad with well developed undulating membrane, posterior end club-shaped. 9. Trypanomorphic Crithidia, parabasal body posterior to nucleus. 10. Schematic drawing of nectomonad after vital staining with Janus green. Figs. 2-9, all $\times 2140$.

The body is surrounded by an extremely thin periplast which encloses the endoplasm and certain permanent cell structures or organelles. In the fixed and stained preparations the endoplasm takes the appearance of a clear fluid supporting many fine granules. In the living condition, however, these smaller granules are not apparent, but instead one may find larger refractile granular bodies supported in a clear fluid medium (Fig. 10). These granules, or "chromidia" as they have been called, seem not to be definitely crystalline in structure, nor do they take the appearance of droplets. Their shape reminds one rather of the appearance of fine particles of broken glass as viewed by the naked eye. When stained with Janus green they range in color from blue to red-brown. Either they disappear in the process of making fixed and stained preparations, or they are so greatly disintegrated that they appear as the groups of granules shown posterior to the nucleus in the figures.

While studying the chromidia of fresh preparations in Janus green with the higher powers of the microscope, I have occasionally seen posterior to the nucleus a rather indistinct structure (Fig. 10), if indeed it is a structure. It is rather difficult to distinguish, but at times there seem to be two clear spirally wound streams of fluid which are in continual agitation, due perhaps to the violent lashings of the flagellum. This structure cannot be found in fixed and stained preparations. Perhaps it is merely endoplasmic streaming similar to the phenomenon of cyclosis as observed in other cells. Swellengrebel (1911) has figured an axial uncolored spirally wound line in his Giemsa preparations of *Herpetomonas calliphorae*, which may be homologous with the appearance I have noted in *Crithidia gerridis*. It seems quite probable that this is the rhizostyle of Alexeieff.

Flagellum.—As may be seen in Table I the average distance from the parabasal body to the tip of the flagellum, which is in reality the length of the flagellum, averages about 21.3μ, a fairly constant measurement as compared with other measurements; e. g., the distance from the posterior tip of the cell to the nucleus. The flagellum seems to originate directly from the parabasal body and runs anteriorly just beneath the crest of a thin expansion of the periplast, forming the undulating membrane. Porter, McCulloch and others mention a basal granule or blepharoplast in their Crithidiae, but I have been unable to demonstrate the like in this particular species. The basal granule probably lies embedded within the parabasal body, for the flagellum can in almost all instances be traced until it makes a contact with the parabasal body. Very occasionally the flagellum is slightly thickened at the point of juncture with the parabasal, but no discrete basal granule can be seen (Fig. 5). As further evidence for the invisibility of the basal granule

it is worth while to note here that in his account of *Tabanus hirtus* Patton makes the statement, "I have never seen a basal granule." Similarly, Swingle wrote of *Crithidia melophagi,* "I have never been able to see a granule at the base of the flagellum and separate from the kinetonucleus."

Parabasal body. In the unstained preparations this appears as a refractile rod-like body at the base of the flagellum from 1.0 to 3.2μ in front of the nucleus. It may be either almost perpendicular to the intra-cytoplasmic portion of the flagellum, or inclined at a more or less acute angle. When the parabasal body in fresh preparations is stained with Janus green it seems to lie inside an area of clear fluid, proving that it is not an artefact due to fixation. Various authors have mentioned a definite membrane enclosing this clear area, but neither in the stained nor unstained preparations can this be demonstrated, although the granules of the surrounding protoplasm are often grouped about the edge of this clear area so as to appear somewhat like a membrane in some cases (Figs. 5, 6). The earlier writers, following Woodcock, referred to this structure as the kinetonucleus—hence the order binucleata of Hartmann. Kofoid and also Swezy (1916) have advanced very cogent arguments to prove that it is not a nucleus, and consequently the order binucleata has no justification. They substitute for *kinetonucleus* the term *parabasal body,* proposed by Janicki in 1911. Wenyon, Dobell, and Alexeieff have ceased to use the term *kinetonucleus,* substituting the name *kinetoplast.* Wenyon uses the term *kinetoplast* to include both the parabasal body and blepharoplast or basal granule. Since the name *parabasal body* was proposed earlier than the name *kinetoplast,* there is no valid reason for rejecting it and substituting the latter name which may have more significance, since in the present state of our knowledge we are not sure that this structure is a plastid. Kofoid has suggested that the parabasal body is reserve material, and that its formation is the result of a change from the free living to a parasitic mode of life. Alexeieff, however, has advanced some good reasons why this structure is an organ of glycoplastic function, and that it serves to elaborate certain materials of importance in the activity of the flagellate. As was indicated previously this body stains in *Crithidia gerridis* with Janus green after the fashion characteristic of mitochondria, but it is by no means certain that as Guilliermond asserts, "The mitochondria give rise to the greater part of the secretory products of the cell and are entirely similar to well known plastids." The opposition of Dangeard (1918) to such views is significant here.

That the chemical nature of the parabasal body is not identical with that of the nucleus is easy to establish, for the nucleus takes little or none of the Janus green stain, while it is quite pronounced

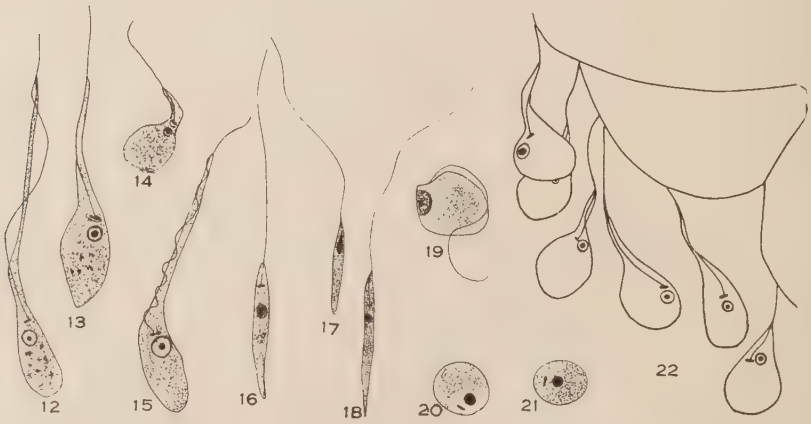
in the parabasal body. Patton noticed that in the case of the Crithidia from *Tabanus hilarius* "the nucleus is a circular mass which stains light pink with Ramanowsky stain. . . . The blepharoplast (parabasal) is a large rod-shaped mass staining deep magenta." Shipley discovered that the parabasal body and certain granules in the cytoplasm of *Trypanosoma lewisi* could be stained with Janus green, and that the nucleus remained unstained.

The nucleus lies just posterior to the parabasal body, and is usually but slightly less in diameter than the width of the cell at that point. Preparations fixed in Bouin's or Shaudinn's fluid show fairly uniformly a "vesicular" type of nucleus; i. e., there is a deeply staining central karyosome, usually almost round (Figs. 3 and 4) but sometimes elongated (Figs. 2 and 8). Infrequently the karyosome is composed of two or three fragments. Surrounding the karyosome is a light space finely and rather diffusely granuled, and the whole is enclosed in a round or oval achromatic membrane. Sometimes this nuclear membrane consists of a number of large clouded granules lying around the periphery (Fig. 3). In such cases the name "membrane" may be misleading, because its constituent parts do not seem to form a continuous enclosure. In general, the nucleus does not differ essentially from that described by McCulloch for *Crithidia euryophthalmi.*

There is a difference, however, between the nucleus of the flagellate found in *Gerris remigis,* and that described in similar flagellates by Patton and Porter. Patton describes eight chromosomes arranged along the circumference of the nucleus. Porter states, "Large chromatin granules, occasionally eight in number, are present. Usually the chromatin of the nucleus is arranged in slightly irregular masses," etc. Whether these authors relied mainly upon Romanowsky stains of the dry smears, or worked with a different species of Crithidia is uncertain. Swellengrebel (1911) described the nucleus of *Crithidia calliphorae* as staining deeply, without any differentiation, and surrounded by a clear halo, due to the contraction of the nuclear substance. He adds, "Similar figures have been mistaken for a nucleus containing a large karyosome, and clearly show how defective is this method of fixing and staining," meaning fixation with corrosive alcohol and staining with iron-hematoxylin. It is exactly this picture which one gets with fixation by Schaudinn's, Bouin's, osmic acid (though not so clearly) and staining with iron-hematoxylin, or by the Benda method. Sometimes the living specimen in Janus green shows, somewhat faintly to be sure, the "vesicular" structure of the nucleus. Staining the fresh cells with methyl green had at least one feature of importance; it caused the nucleus to stand out boldly, showing both the karyosome and the nuclear membrane.

McCulloch discovered in *Crithidia euryophthalmi* a cone shaped structure (parabasal rhizoplast) between the basal granule and the parabasal body, and a rhizoplast connecting the nucleus with the basal granule. Since in *Crithidia gerridis* the flagellum seems to take its origin directly from the parabasal body, there can be found no structure comparable to a parabasal rhizoplast. Very rarely one finds a specimen showing a fine line between the nucleus and the parabasal body, which reminds one of the nuclear rhizoplast seen by McCulloch (Fig. 4).

Haptomonads. These are the attached forms which sometimes line the wall of the first stomach in great profusion. They must be grouped into two classes:



Figs. 12, 13, 14.—Haptomonads from first stomach. 15.—Haptomonad form found in rectum, showing dividing parabasal body and new flagellum beginning to grow out therefrom. 16, 17, 18. Small nectomonad forms with long flagella from rectum. 19. Encysting form. 20, 21. So-called cyst forms. 22. Outline drawing of cross section showing haptomonads attached to intestinal wall. Figs. 12-22, all × 2140.

(a) Those which are similar morphologically to the nectomonads, except that they are smaller in some cases.

(b) Those which have the same general structure of the organelles as the nectomonads but differ in size and shape (Figs. 12-15). They vary in length from 7.4 to 25.0μ, and in width from 2.0 to 3.3μ, being thus somewhat wider than the nectomonad forms. The posterior end is rounded, giving the flagellate an appearance quite different from that of the long graceful nectomonads. These forms attach themselves in great masses to the tips and sides of the tall epithelial cells (Fig. 22).

Patton and Porter mention finding cysts in the crop of the insects, and they describe a process of development from the cyst to the flagel-

late stage. Almost any interpretation can be placed upon dried preparations of the haptomonad forms stained by the Romanowsky method, since some show either no flagellum or a faintly staining one. Iron-hematoxylin preparations of these same forms show a well developed flagellum in all instances. I believe that most of the cyst forms which Patton and Porter found in the crop were in reality degenerated haptomonads or yeasts.

Second Stomach, Third Stomach, Ileum.—The third stomach and ileum rarely contain more than a comparatively insignificant number of flagellates. In some heavy infections of the first and second stomach, sections of the digestive tract will contain at most only a few nectomonads swimming in their contents and a few haptomonads attached to the intestinal wall. The anterior end of the first stomach, however, often swarms with flagellates which resemble those in the first stomach. Generally speaking, the infection becomes scantier as one proceeds backward from the anterior end of the first stomach toward the rectum.

Rectum.—Minchin and Thompson (1915) found that *Trypanosoma lewisi* might permanently establish itself in the rectum of the rat flea after the infection had disappeared from the anterior portions of the intestine. McCulloch (1919) found that there was a permanently established phase of the life cycle of the parasite in the pyloric expansion of the lupine bug corresponding to the established rectal phase of *Trypanosoma lewisi*. In the water-strider one finds few flagellates attached to the wall of the rectum, so that it seems improbable that rectal haptomonads are a part of the life cycle. However, a few haptomonads (Fig. 14) and nectomonads are present which have probably passed down from the anterior part of the intestine.

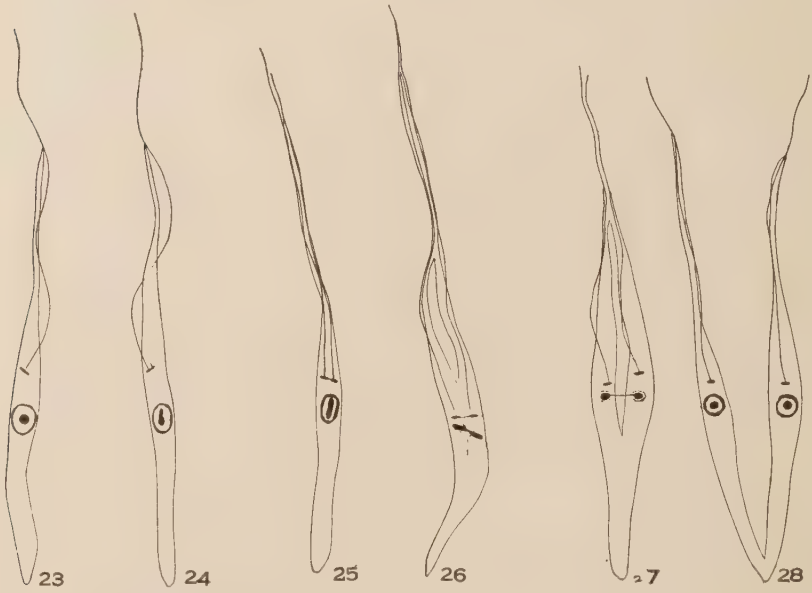
The rectum often contains another form of the parasite which is occasionally encountered anteriorly. It is a long free-swimming form, very narrow (0.7 to 1.2μ in width), with a short body and a long flagellum, making the total length from 21 to 38μ (Figs. 16, 17, 18). On account of their small size it was impossible to study accurately their nuclear structure. The spirochaetiform flagellates which Porter figures from the rectum resemble these in that they are very narrow, but the cell proportions are quite different.

The structure of this type of flagellate is remarkably like that of Herpetomonas. It is too small to determine whether or not there is a rudimentary membrane. That it is a stage in the life history of this Crithidia is certain, however, for no infections are to be found where there are not also crithidial forms present, and all gradations of the flagellate between this type and the large flagellates may be found. Here also are found the encysting and cyst forms already described by Porter (Figs. 19, 20, 21). The cysts measure from 1.5 to 4.0μ in diameter. A definite cyst wall is lacking. Whether or not

this is the infective stage has never been experimentally proved under conditions which would eliminate the presence of all other forms of the parasite. Wenyon was able to cultivate herpetomonads from the feces of *Pulex irritans* which had been allowed to dry for 24 hours. This might be taken as evidence that these rounded forms are the actual resistant infective forms.

MANNER OF MULTIPLICATION

No method of multiple fission corresponding to the multiplication spheres of Minchin and Thompson, or to the somatellas of McCulloch was found. Sections of two entire heavily infected intestines and



Figs. 23-28.—Outline drawings of dividing forms arranged in series to show process of division.

study under the microscope of scores of fresh intestines failed to disclose intracellular phases of any nature in the life history. The very interesting process of endogenous budding in *Crithidia euryophthalmi* discovered by McCulloch was carefully searched for with negative results. Appearances simulating the multiplication rosettes of Patton and Porter were found, but this method certainly does not play any prominent part in the multiplication of the parasites.

Among all types of the Crithidia present in an infection the prevailing method of multiplication is binary division. Only a relatively few specimens in the advanced stages of division are to be found, but a large proportion of the number in one stained smear will show the

beginning processes of division. The first apparent indication of division is a dividing parabasal body (Figs. 5, 15 and 24). The two halves move away from each other, connected at first by a fine filament (Figs. 6, 7, 25 and 26). Apparently the old flagellum remains with the half to which it is attached, and the new flagellum arises from the other half. Whether the flagellum divides or splits has been the subject of much controversey. The new flagellum is so precocious in its growth, and follows so closely the course of the old one that it is difficult to follow the process exactly. Such appearances as in Figure 15 suggest that the new flagellum is a new outgrowth.

Meanwhile the karyosome of the nucleus commences to lengthen in the direction of the long axis of the cell (Figs. 5, 24 and 25). Next it swings to an angle, sometimes taking a position at an angle of 90° to its former position (Figs. 6, 7, and 26). A constriction appears at about the middle of the elongated karyosome as the two halves move away from each other (Figs. 6, 7, 26 and 27). At this stage the nuclear membrane becomes indistinct, the clear area surrounding the karyosome diminishes so that the karyosome seems to lie almost in contact with the granular endoplasm (Figs. 7, 26 and 27).

While the nuclear division is advancing to this stage the body of the flagellate widens quite noticeably in the vicinity of the nucleus. A clear groove interposes between the members of the pair in process of development, across which a dark staining filament connecting the two members of the dividing karyosome may run (Figs. 7, 26, and 27). As this groove widens it extends anteriorly until both anterior ends are free (Fig. 28). Usually before the division posteriorly is complete the two individuals have their nuclei and undulating membranes completely reconstructed. The two flagellates then pull apart posteriorly.

There is no indication that the granules posterior to the nucleus (chromidia) divide. They go passively with the half of the body in which they are embedded.

In the division of the nucleus there is no indication of a centrosome or attraction sphere. The karyosome seems to supply the activity needed for a nuclear division. If one regard the karyosome as the active element in the division one may consider the process one of promitosis.

SUMMARY AND CONCLUSIONS

1. The North American water-strider, *Gerris remigis,* is parasitized by a Crithidia. The infection rate at Cold Spring Harbor was 20.5 per cent.; in the vicinity of Baltimore, 2.5 per cent.

2. The infection ranged from extremely light to massive. The parasites occur in the intestine from the first stomach to the rectum.

3. In the first stomach are found the nectomonads, or free-swimming forms, and the haptomonads, or attached forms. Measurements

of 100 nectomonads are tabulated, and the endoplasm and organelles described. The morphology of the haptomonads is essentially that of the nectomonads, except that the former are shorter and the posterior end is wider and more rounded.

4. The second stomach, third stomach, and ileum have all the forms in the first stomach, but in smaller numbers.

5. In the rectum are nectomonads and haptomonads from anterior part of intestine. A very narrow nectomonad with a long flagellum predominates the cysts and encysting forms.

6. The only process of multiplication observed was binary fission.

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OBSERVATIONS ON AN INTESTINAL FLAGELLATE OF TROUT

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During the spring of 1922 fingerling trout at two of the Bureau of Fisheries hatcheries were found to be badly infected with an intestinal flagellate which was apparently causing serious injury. While the investigation of this parasite is not completed the results obtained are believed to be of sufficient interest to justify the publication of a preliminary paper.

The parasite was often present in enormous numbers, in some cases almost the entire contents of the intestine in the infected region being composed of a squirming mass of flagellates. Both brook trout (*Salvelinus fontinalis*) and rainbow (*Salmo shasta*) were infected, the infection being somewhat more severe in the latter species. The flagellates were most abundant in the anterior end of the intestine, just behind the stomach in the region of the pyloric ceca. Unless very numerous they were usually found in numbers only in this region but in severe infections occurred throughout the entire length of the intestine. In some cases the flagellates were found in large numbers in the ceca but this condition appears to be exceptional.

This parasite was described by Dr. Emmeline Moore under the name *Giardia salmonis* in a paper read at the 1922 meeting of the American Fisheries Society. Miss Moore has since advised the writer in a private communication that she now believes the flagellate should be assigned to the genus Octomitus rather than Giardia. She has very kindly sent me some of her material for comparison and there can be no doubt that we have both been dealing with the same species.*

The parasite is very similar to the one reported by Moroff (1903) from the intestine of rainbow trout under the name *Urophagus intestinalis* since he believed it to be the species previously described by Dujardin from the intestine of Amphibia. More recently this species has been studied by Schmidt (1920) who also concludes that it is not specifically distinct from the well known amphibian parasite but considers it a subspecies, *Octomitus intestinalis truttae*. The fact that both the European and American forms occur in the same host species is strong presumptive evidence that they are identical. However, Schmidt's description and figures differ in several essential respects

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* Dr. Moore's description of the parasite published in the Twelfth Annual Report (1922) of the New York State Conservation Commission, pp. 69-76, was received too late for consideration in this paper.

from the American form. This is especially true of the axostyles and cytostome. The former are represented as being much broader and more rigid in the European form. Furthermore, there can be no doubt that the flagellate studied by Miss Moore and myself is specifically distinct from the amphibian parasite, *Octomitus intestinalis* (Duj.).

Under the circumstances it is believed that Moore's name, *Octomitus salmonis* should stand since there is no conclusive evidence that the parasite described by Moroff and Schmidt is specifically identical with the one occurring in American trout.

DESCRIPTION OF FLAGELLATE

The form of the body is quite variable but is usually ovoidal or pyriform, rounded anteriorly and tapering gradually toward the posterior end which is more or less distinctly truncated (Figs. 1 to 4). There is also considerable variation in size, the majority being about 10 to 12μ long by 6 to 8μ in breadth. On the ventral side at the anterior end is a very mobile disk-like structure, the so-called cytostome. While evidently an attachment organ the cytostome is not as large as in Giardia and only slightly concave. Behind the cystostome a slight ridge can usually be distinguished extending for some distance along the mid-ventral line.

As in other hexamites there are four pairs of flagella three of which rise close together at the anterior end while the origin of the fourth is at the extreme posterior end. The latter are about twice the length of the body while the anterior flagella are somewhat shorter. The protoplasm is finely granular and often contains numerous small vacuoles. There is no distinction between ectoplasm and endoplasm and no distinct periplast membrane. A pair of slender hyaline axostyles can usually be seen extending the entire length of the body.

These are the only structures which can be made out in fresh or unstained material. For the study of the internal structure wet smears were fixed in Schaudinn's and other standard fixing fluids and stained with iron hematoxylin. Smears fixed in Zenker's fluid and stained by Mallory's eosin and methylene-blue method were of considerable value in supplementing the iron-hematoxylin preparations.

The nuclei are a pair of rounded or ovoid bodies situated a short distance from the anterior end on each side of the axostyles, and just dorsal to the cytostome. They stain deeply and uniformly with chromatin stains. Just anterior to the nuclei is the blepharoplast complex composed of several deeply staining granules attached to the anterior ends of the axostyles. It sometimes appears as a single, somewhat irregular body but more often can be plainly seen to be composed of two or three pairs of granules, one component of each pair being attached to each axostyle. In all probability there are always three

pairs of granules separated by varying distances and sometimes so close together that they cannot be distinguished as discrete particles.

A short distance behind the blepharoplast and between the nuclei is a rather indefinite mass of granules which usually take the chromatin stain. This finely granular material appears as a deeply stained band lying between the axostyles (Fig. 3). It is probably the homologue of the parabasal bodies of Giardia but lacks the constancy of form usually attributed to these structures. Near the posterior end is another mass of chromatic material which often forms one of the most conspicuous features in stained preparations. This is also composed of fine granules and usually appears as two to four conspicuous bands extending along the axostyles about one-third of their length (Figs. 3 and 4). The varying number of chromatic bands is simply due to variations in the position of the axostyles. When the latter are close together the chromatic material is squeezed out from between them and appears as a double band, one on each side of the axostyles. When the axostyles are a short distance apart the chromatic material forms three bands, the most conspicuous lying between the axostyles. If the axostyles are separated still farther the chromatic granules appear as a distinct band along each side of both axostyles. Usually these chromatic bands form distinct ridges projecting above the general surface of the body with well defined grooves between at the bottom of which lie the axostyles. Both anterior and posterior chromatic bands are usually quite conspicuous but are sometimes difficult to distinguish, especially in strongly decolorized preparations. They are particularly well brought out by Mallory's eosin and methylene-blue method.

The axostyles are a pair of slender rods, usually staining only slightly or not at all, which extend from the extreme anterior end to the extreme posterior end of the body but normally do not project at either end. The anterior ends, to which are attached the blepharoplast granules, lie at the anterior margin of the cytostome. From the blepharoplast the axostyles extend posteriorly and ventrally reaching the surface just behind the cytostome where they form a median ridge which is often quite prominent (Fig. 4). At the posterior end the axostyles curve dorsally, forming the caudal grooves already referred to, and finally terminate in the long caudal flagella. While this is believed to be the typical arrangement of the axostyles they often present a quite different appearance. They are evidently very flexible and easily displaced by contractions of the body. Sometimes they may be some distance apart or greatly curved, while in still other cases they may cross each other, especially near the posterior end.

The cytostome is often a very conspicuous feature in stained preparations. This is due to the fact that it contains numerous fine granules which stain deeply with chromatin stains. In fact the

cytostome stains so deeply that it is often difficult to distinguish the nuclei, which lie just above it, as separate structures unless the smears are strongly destained. The coloration is, however, not uniform but is much more intense near the posterior and lateral margins. The anterior portion stains less deeply or not at all. With the eosin and methylene-blue method this region stains a deep red. No peristomal fiber could be distinguished and if present must be hidden by the chromatic granules which are especially abundant around the margin. The cytostome may appear as a single slightly concave disk (Fig. 4) but usually shows a more or less distinct bipartite structure. In some cases, especially in strongly contracted individuals it is divided into two entirely separate structures.

THE INTRACELLULAR STAGES

In addition to the flagellates which occur so abundantly in the lumen of the intestine an intracellular parasite is always present in infected fish. This parasite is found in the epithelial lining of the pyloric caeca and anterior end of the intestine. While usually very different in appearance from the flagellate in the intestinal cavity there is strong evidence that it is an intracellular stage of the same organism and forms an essential part of the life cycle. The intracellular parasite is often present in large numbers and may cause an extensive inflammation of the intestine terminating in the death of the host. Strangely enough the intracellular parasites while often quite common in the cecal epithelium of fish at the hatchery were found in greatest abundance in fingerlings which had been shipped in cans to Washington. The fish were on the road overnight and a large percentage died in transit while most of the survivors were in poor condition when received. The ceca and intestine of these fish when sectioned showed in almost every case an excessive infection with intracellular parasites. In some individuals they were so numerous as to practically destroy the epithelium in places.

The intracellular parasites appear in various forms which are evidently stages in a developmental cycle. They are usually found in the distal half of the cell surrounded by a distinct vacuole (Figs. 7 and 8) which is probably largely the result of shrinkage. While the parasites are at first much smaller than the infected cells they increase rapidly in size causing hypertrophy of the cells which are probably in all cases eventually destroyed. The most common form is a more or less elongated cell with a large vesicular nucleus at one end (Figs. 16 and 17). This nucleus usually contains about six irregular masses of chromatin which vary considerably in size. In some cases, especially in fish which contain few or no flagellated forms, the chromatin masses are less distinct and there is a marked tendency for the chromatin to collect on the nuclear membrane (Fig. 17). Larger cells are not

infrequently seen which differ from those described chiefly in size and in the fact that the nucleus contains about twelve chromatin masses instead of six (Fig. 9). A third type of cell is shown in Figures 5 to 7. These cells are much smaller than those previously described and the nucleus is often near the center instead of being at one end. They evidently develop into the larger cells previous'y described, although the details of the process have not yet been worked out.

The increased number of chromatin masses in the large cells (Fig. 9) is evidently in preparation for division which is shown in Figure 10. The daughter cells formed by this division are unsymmetrical and associated together in a very remarkable manner. One cells is rounded and fits into a cup-shaped depression in the other cell (Figs. 11 and 12). Sometimes the enveloping cell may be greatly flattened and almost entirely enclose the other. Pairs of cells such as those figured are not uncommon and can certainly not be looked upon as abnormal. At first it was thought that they might represent a stage in a sexual process but a careful study of a large number of such cells has convinced me that they are the result of division and have no sexual significance. This division is quickly followed by others until a group of six to eight small cells is formed (Figs. 13 to 15). Apparently after each division one daughter cell is at first more or less enclosed by the other (Fig. 13). However, the details of the process are very difficult to make out since at this stage the cells are so closely associated that in most cases it is impossible to distinguish the limits of the individual cells.

The evidence at hand indicates that the further development of these cells may be along two quite different lines. Part grow rapidly and in turn divide into a number of cells as just described. Others instead of again undergoing schizogony have a very different history. In such cells the nuclei become metamorphosed and eventually contain two large rounded masses of chromatin symmetrically arranged and of approximately equal size (Figs. 17 and 18). In addition to the chromatin which forms the paired chromatic bodies there is always a considerable residuum which is at first quite noticeable but gradually loses its affinity for stains. This residual chromatin is often especially abundant near the middle of the nucleus. At a somewhat later stage the nucleus degenerates (Figs. 20 and 23) and becomes indistinguishable leaving the chromatin masses as a pair of rounded compact nuclei near one end of the cell.

There remain to be considered certain stages which are probably somewhat abnormal but nevertheless appear to be of considerable significance. In a number of instances cells have been observed in which two or three small masses of chromatin could be distinguished

by the side of the nucleus. In several instances the chromatic bodies formed a small but distinct nucleus as shown in Figures 21 and 22. The relation of the two nuclei strongly suggests that the small nucleus is formed by chromatin which has been extruded from the larger. In a number of cells the small nucleus was at some distance from the larger (Fig. 22). Figure 23 probably represents a later stage in the development of such a cell.

What then is the evidence for considering the intracellular parasites and the free living flagellates simply different stages in the development of a single species. In the first place a careful study of Figures 17 to 24 show that here is a series of stages which form a gradual transition from the intracellular forms to the typical flagellates. These transitional forms are not exceptional but may be found in considerable numbers in badly infected fish. The only stages which can be considered rare are those represented in figures 20, 23 and 24. It is not difficult to understand why these stages should occur only exceptionally. The organism has reached its extreme development as an intracellular parasite and is about to emerge into the lumen of the intestine. Under these conditions it is only natural to assume that the transition would take place rapidly. Practically the only difference between the latest stage of the intracellular form and the mature flagellate lies in the entire absence of locomotor organs in the former. In no case has it been possible to distinguish any evidence of flagella in the intracellular forms and in only a few instances has it been possible to make out any trace of the axostyles. While it is realized that this fact furnishes an argument against considering the two forms specifically identical it is not believed that it is a serious one. In the first place it seems highly improbable that the parasites would remain long within the cells after the locomotor organs have developed. Furthermore, both flagella and axostyles are very delicate structures and can only be distingiushed in exceptionally well fixed and stained material. I have been unable to make out the flagella in the free-swimming forms in sectioned material except in a very few instances and even the axostyles are often indistinguishable in such cases.

It is interesting to note that if Figures 17, 18, 19, 20 and 24 represent successive stages in the metamorphosis of the intracellular parasites, as is believed to be the case, one can trace all the deeply staining structures of the flagellated form back to the nucleus of the intracellular parasite. The nucleus is always located at one end of the cell which later becomes the anterior end of the flagellate. The blepharoplast, the deeply staining granules of the cytostome and the parabasal body are apparently derived from the residual chromatin which is left within the original nucleus after the formation of the paired daughter nuclei. It is also probable that the chromatic bands at the posterior ends of the axostyles are of nuclear origin. Figures 21 to 23 indicate that

the chromatin extruded from the nucleus passes to the opposite end of the cell where the chromatic bands make their appearance a little later. The most logical explanation of these stages would appear to be that a similar extrusion of chromatin takes place in all cases but that normally the extruded chromatin quickly disintegrates and loses its affinity for chromatin stains. Eventually it collects around the axostyles at the posterior end and again takes the chromatin stain.

In further support of the contention that one is dealing with different stages in the life cycle of the same species it should be pointed out that in all cases which have been carefully investigated the intracellular forms have been found along with the free-swimming flagellates. Both forms have been found associated in fish from widely separated localities, one in West Virginia, another in Tennessee and two in New York State. The latter record is based on material kindly sent me by Dr. Emmeline Moore. These are the only localities from which material has been available up to date.

It is also significant that in general there is a close parallelism in the abundance of the two forms. In no instance have the intracellular stages been abundant except in fish in which there were also great numbers of typical *Octomitus* in the intestinal cavity. It is true that in some cases the intracellular parasites have been found in fish in which no free-swimming parasites could be demonstrated but in all such cases they were comparatively rare and in some instances were found only after long and laborious search. In none of these cases did the parasites appear to be multiplying rapidly, practically no division stages were found, and they appeared to be in a more or less dormant condition. The most probable explanation appears to be that the intracellular stages are more resistant than the free-swimming flagellates and may persist after the latter have disappeared. There is evidence that when the fish are weakened or subjected to unfavorable conditions the development of the intracellular forms is stimulated and free-swimming flagellates soon make their appearance in the intestine.

It should also be pointed out that no protozoan parasites other than the two forms described above were found in any of the infected fish. If the intracellular parasites are not Octomitus it is certain that they never occur in any numbers in the lumen of the intestine. It is true that at certain stages they somewhat resemble coccidia but no evidence has been found of sporulation or of the formation of gametes. Furthermore, they do not exhibit the staining reactions so characteristic of coccidia which are notoriously difficult to stain.

As to the stage at which the parasites enter the epithelial cells I have, at present, no definite knowledge. They are probably transmitted from one fish to another by means of cysts which germinate in the stomach or intestine. After leaving the cyst it is not improbable

that the organisms penetrate the epithelium and multiply for a time by schizogony before developing into the flagellated form. However, for the present this must remain pure conjecture.

EFFECTS OF THE PARASITE ON THE HOST

My attention was first called to these parasites by complaints that fingerling trout from the West Virginia hatchery did not stand shipment as they should. The loss during the first twenty-four hours was not excessive but if the fish were in transit for a much longer period the loss was exceptionally heavy. In an attempt to discover the cause of this exceptional mortality fish were shipped to Washington and examined shortly after their arrival. It was found that in many instances the anterior end of the intestine was badly inflamed and filled with enormous numbers of flagellates. In most cases they had invaded the ceca but were not as abundant here as in the intestine. When sectioned the inflamed portion of the intestine and pyloric ceca were found to contain intracellular parasites in such numbers that a large proportion of the epithelial cells were destroyed and this undoubtedly caused the inflammation. It is very doubtful if the free-swimming forms in the intestinal cavity are by themselves capable of producing a serious inflammation. The hatchery was visited early in July and at this time many of the fingerlings were found to harbor the flagellates in enormous numbers, but the intestine showed no signs of inflammation. In all such cases, however, subsequent examination of sections of the intestine and ceca disclosed the fact that the intracellular stages were only moderately abundant.

It is interesting to note in this connection that Moroff found that the intestines of trout infected with the flagellates were badly congested but was uncertain as to whether they were the cause of the inflammation. Miss Moore states that the parasites have caused several severe epidemics in the New York State hatcheries. On the other hand Schmidt concludes that the organism is a harmless commensal. He was, however, apparently dealing chiefly with larger fish and it is very probable that the parasite does not seriously injure the trout after they reach a length of 3 or 4 inches. The evidence at hand indicates that it is distinctly a disease of the fry and young fingerlings. At this stage the parasites may seriously interfere with their growth and under certain conditions cause an enteritis which may terminate fatally.

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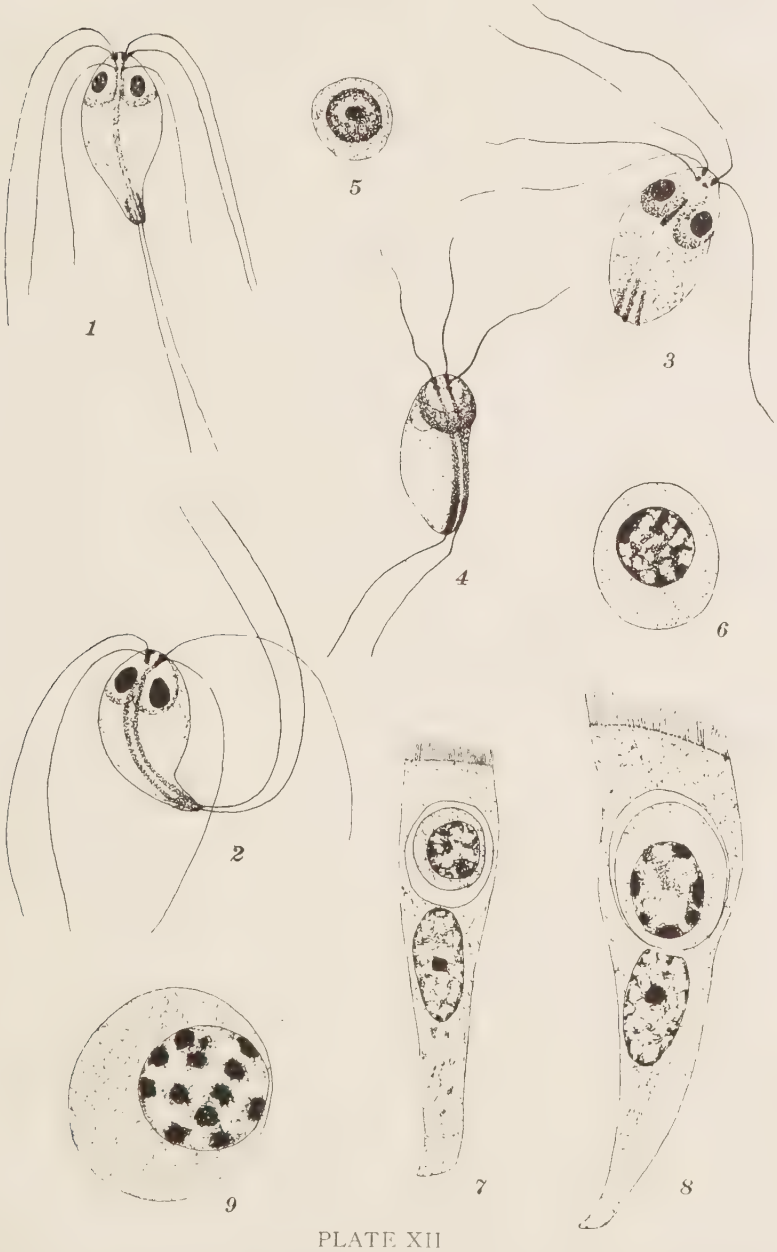

PLATE XII

Figs. 1-4.—Adult flagellates viewed from the ventral side. Only those flagella which could be plainly distinguished are shown in the figures. The nuclei are unstained in Fig. 4.

Figs. 5-6.—Small intracellular forms. Figs. 7-8.—Epithelial cells with enclosed parasites. Fig. 9.—Large intracellular form.

Figures 7-8, × 1640. All other figures × 2400.

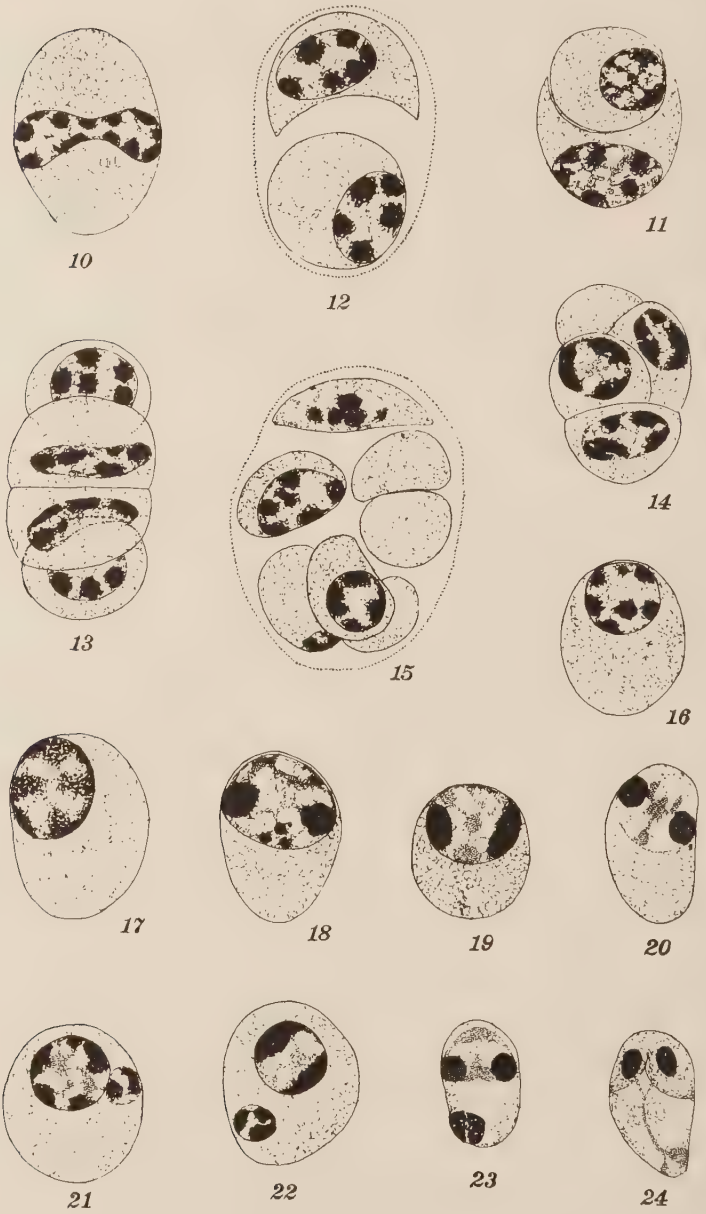

PLATE XIII

Fig. 10.—Early stage in division of intracellular form. Figs. 11-14.—Stages in sporogony. Fig. 15.—Late stage in sporogony. Figs. 16-20.—Successive stages in development of flagellated form. Figs. 21-22.—Extrusion of chromatin from nucleus. Fig. 23.—Later stage than Fig. 22. Extruded chromatin at posterior end. Fig. 24.—Late stage of intracellular form shortly before leaving epithelium. All figures × 2400.

THE EFFECTS OF THE BITE OF *LATRODECTUS MACTANS* FABR.

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References in literature, popular as well as scientific, concerning the supposed effects of the bite of the so-called Black Widow, are very commonly met with. These accounts are as numerous as they are varied. It is perhaps not underestimating the value of this literature to say that it is all circumstantial evidence. Some of the evidence is fairly good as such, some of it obviously not to be relied upon.

Kobert has made extensive experiments with the poison of the European species, *L. erebus,* using an extract that presumably contained the active principle of the poison. Although his results show conclusively that the spider possesses a substance that acts as a virulent poison to various small animals, such as cats, dogs, guinea pigs, etc., yet they do not, so far as I can see, tell us anything in regard to the effect of the bite of that spider. To my knowledge, there is no record of a successful attempt to obtain direct evidence on the effects of the bite, either on animals or man. This paper is an account of some observations * on the effect of the bite of *Latrodectus mactans* on young rats and on man.

The effect on rats. Six white rats, about one month old, were used. For convenience, they are designated A, B, C, D, E, F. Three female spiders were used in these experiments and they have been named A, B, C.

A somewhat serious difficulty that has been met with by several workers is that involved in inducing spiders to bite. Since Latrodectus does not attack its prey with its fangs, but wraps it many times around with heavy strands of viscid silk; it is not disposed to bite as readily as are, for instance, the funnel web weavers which were used for comparison.

After a number of attempts, I learned that the spiders (*L. mactans*) bit very readily if they were fed a certain time previous to the attempted test. The spiders were fed on half grown nymphs of various species of green grasshoppers (Mermeria), since these were most conveniently secured and close by the laboratory. If the spiders were fed on the day before the test was to be made, they practically refused to bite. If they were left without food for a week before the test, they were not easily induced to bite. If they were fed 48 hours before the test,

* Mr. A. R. Garlington, a student in the university, rendered valuable assistance in making the tests and taking the observations.

they bit as soon as placed for the test, and held on till they were removed by force. This made it possible to have the poison administered in small as well as large doses and observe the relative effects. The rats used in these tests were about one month old. On the inside of the left hind leg, the hair was clipped fairly close and here the spiders were applied and induced to bite.

In applying the spider for the bite, it is held between the thumb and first two fingers of the right hand. Because of the very smooth and globular abdomen, it is necessary to have a camel's hair brush, or pencil, in the other hand and by means of this keep the spider in the proper position. In the first tests the camel's hair brush was used to tease the spider and thereby induce it to bite. This was futile. It was soon found that the spider, when at all in the mood to bite, is most easily induced to do so by putting the fangs on the selected spot, and gently moving the spider from side to side. By this procedure, one or the other of the fangs will catch hold in the skin and the spider is thereby induced to implant both fangs as deeply as possible.

The first test was made on July 4. Two young rats, about one month old, were used. Rat A was bitten twice by Spider C; Rat B was bitten by Spider B. The following is a record of the observations essentially as they were recorded at the time.

Rat A and Spider C.—11:02 A. M. Rat bitten twice on inside of left hind leg. The first bite produces a reddening of the skin. When placed in cage, the rat licks over the bitten place, lifts the leg. A small drop of blood gathers where the fangs penetrated the skin.

11:05. Continues to lick inside of leg.

11:06. Appears sick, nervous, tries to crawl under excelsior, holds up leg.

11:10. In moving lifts leg far off the floor.

11:20. Bitten place has purplish color, rat tries to hide under paper on bottom of cage.

11:22. Presistent attempt to crawl under paper.

11:30. Eyes still wide open, but head is bent under to reach hind leg. Keeps on licking over bitten place.

11:38. Humps up, continues to lick leg.

11:40. Eyes nearly closed.

11:42. Humps up as though trying to stand on its head; jerks head as if in convulsions.

11:48. Moves spasmodically, turns several times around, sits still a moment, very much humped up, eyes practically closed.

11:52. When picked up, rat opens eyes wide, then moves more actively for a while, then settles to former attitude, jerking frequently, tries to climb, licks leg again.

12:05 P. M. Appears to have convulsions, jerks more than before, walks about with eyes closed, holds leg clear off the floor.

12:10. Walks unsteady, rises high on toes and falls. Gets up slowly and continues trying to walk.

12:12. Stands almost on its head, and falls.

12:17. Very restless and nervous, puts claws in screen of cage, has difficulty in withdrawing them.

12:35. Head turned clear under, resting on its face. Rolls up in a ball, raises up, then falls over. Rubs leg with paws vigorously.

12:50. Sits very still with face on floor; tries to hump up closer.

1:00. Eyes open, walks at intervals, limps, but uses the leg.

1:25. When picked up appears wide awake; when put down sits fairly still with nose in corner of cage. Jerks occasionally, not so often as before.

2:00. Sits fairly still, eyes wide open, turns around; when disturbed, walks about, using the left leg.

2:25. Sits humped up, jerks as if in convulsions.

3:00. Fairly quiet, moves occasionally, jerks and licks left hind leg.

3:30. Sits up when aroused, opens eyes, humps up, then apparently settles to sleep, or state of coma.

4:00. Asleep or state of coma, rouses, licks leg, then settles down again.

4:15. Moves rapidly, stops to lick leg, then moves again, seems to be in considerable pain.

5:00. Drinks, sits up, refuses food.

5:30. Sleeps with head under body, when aroused it seems restless, licks leg. Leg slightly swollen, bitten spot slightly inflamed.

6:00. Sleeping (in coma?); refuses food.

6:30. Continues sleeping, when aroused walks nervously, scratches side, using left leg.

7:00. Restless, eyes closed. When aroused, walks on all four feet, eyes wide open, seems quite alert.

10:00 P. M. Apparently quite normal.

Rat B and Spider B.—This rat was bitten at 10:50 A. M. It behaved in a manner very similar to A, except that it did not hump up so much. At 7 P. M. it was walking around on all four legs, and apparently had practically recovered from the effect of the bite.

Another test was made on July 9. The rats used in the first test were disposed of. The ones used here are designated by C and D. The spiders used here are A and B. They had been fed in the forenoon of the preceding day. Apparently for this reason, as was learned later, they could not be induced to bite the rats as they did in the previous test. No record was kept on the feeding preceding the first test. Only a brief summary of the symptoms of the rats needs to be given here.

Rat C was bitten by Spider A at 10:10 A. M. The fangs entered the skin, but not sufficiently to leave any marks. The symptoms following the bite were similar to those exhibited by Rat A; but in a much milder form. By 2:10 P. M. the rat had practically recovered.

Rat D was bitten at 10:15 A. M. Spider B seemed to bite willingly, but probably did not insert fangs deeply. The symptoms in this rat corresponded very closely to those of Rat C.

On July 10, the following day, the test was repeated, using the same spider on the same rat. At this time the spiders bit freely and well. Since the symptoms were rather mild, and similar to those already related, they are but briefly summarized.

Rat C.—8:15 A. M. Rat C. was bitten by Spider A. The spider inserted the fangs well, and held on so that it had to be removed by force. The fangs remained inserted for several seconds.

8:35. The spot where the fangs penetrated shows considerable reddening. Rat sits humped up, eyes closed.

9:00. Walks on all four feet, protects the left hind leg but slightly.

9:45. Appears almost normal, feeds.

11:00. Apparently sleeping, seems perfectly normal.

4:15 P. M. Eats heartily, apparently quite normal.

Rat D.—8:20 A. M. Spider B bites rat, implanting fangs deep enough to draw some blood. Fangs allowed to remain inserted for several seconds.

8:30. Surface near bite red, apparently some inflammation.

9:30. Seems very sick, lies stretched out, chin resting on edge of cage.

9:45. Eyes wide open, refuses to stir when cage is shaken.

10:05. Eyes half closed, breathes spasmodically.
11:30. Walks on all four legs.
2:00. P. M. Seems much better, eyes open, refuses water.
4:15. Resting on belly, refuses food.
July 11.—9:00 A. M. Feeds and takes water. Leg shows inflammation on bitten place. (This began to fester in a few days.)

The effects of the bite of Latrodectus apparently were not producing any such results on the rats as would indicate a very virulent poison. Therefore, it seemed desirable to compare these results with those following the bite of a supposedly harmless spider. For this was selected a funnel web weaver, *Agelena naevia* Walck., very common about here. In one respect this spider is very suitable for this kind of experimentation. It is easily held, and bites fiercely as soon as it has an opportunity. When it bites, there is no doubt about it, it draws blood at once. The rats used in this test were E and F. Two funnel web weavers were used.

Rat E.—10:25. Spider bites rat vigorously twice.
10:50. Rat sits slightly humped up, eyes closed.
11:05. Seems nervous, eyes partly closed.
11:30. Eyes closed, when aroused, seems quite alert, walks and uses left leg freely.
2:10 P. M. Resting with eyes open. Entirely normal.
Rat F.—10:35 A. M. Large specimen of funnel web weaver bites rat twice, implanting fangs deeply and holding on. The fangs are allowed to remain for several seconds, and the spider is then withdrawn with some difficulty.
10:40. Rat holds up leg, licks wound.
11:00. Sits with eyes partially closed and when aroused seems normal. Skin around punctures shows no purple discoloration.
2:10 P. M. Resting on belly, eyes open, walking. Uses the leg freely.

Although the bite of *Latrodectus mactans* produced only moderately pronounced symptoms, yet these effects are clearly different from those following the bite of the funnel web weaver. While the latter bit well through the skin, the poison, if any of it entered the system, apparently did not produce the slightest effect on it.

IMMUNITY TO THE EFFECTS OF THE POISON OF LATRODECTUS

When as described later in this paper the second bite on myself produced such striking results, after those following the first one were scarcely perceptible, it was suggested to me that possibly the first small dose acted as a sensitizing agent. To test this, the rats C and D that had already been bitten twice were kept, and subjected to further observations.

July 16. Rat C.—9:25 A. M. Rat C bitten by Spider A. Spider did not bite well, at least did not hold on well. (The spiders had not been fed for a period of 8 days.)
Observations were recorded at short intervals until 1:30 P. M., when the rat seemed to behave in an entirely normal manner. The symptoms resembled those already recorded for previous tests; but were not so clearly defined.

Rat D.—9:27 A. M. Rat bitten by Spider B. Spider seems to bite promptly (this spider, by disposition, always seemed more inclined to bite than the others). The spot where the rat was bitten previously had developed into a sore spot with some pus in it.

The symptoms were like those of C, consisting of much licking of the hind leg, and more or less nervousness and restless moving about.

A final test was made on July 23. Rats C and D were again bitten by Spiders A and B, respectively. Following this bite, the rats exhibited no other symptoms than licking of the inside of the bitten leg. They remained entirely alert, fed at short intervals, climbed the screen walls of the cage, etc.

From these observations, it seems that in rats, immunity to the effects of the poison of Latrodectus mactans is developed rather rapidly. Three doses here produced a degree of immunity that prevented the appearance of any visible symptoms following the bite.

EFFECT OF BITE OF *LATRODECTUS MACTANS* ON MAN

Since the bite of this spider produced no very serious results in the young rats, as observed on July 4, it seemed desirable when making the second test on rats, to make the test also on man. It is doubtless a far-fetched conclusion that if the poison produces mild effects on young rats, it will not be dangerous to man; yet this conclusion I found quite safe in the case of the tarantula (*Eurypelma steindachneri*).

Accordingly, on July 9, when the second test on the rats was made, I attempted to induce Spider C to bite me on the inside of the basal joint of the small finger of the left hand. In spite of all coaxing and teasing, it made not the slightest attempt to bite. It deposited a considerable amount of liquid (possibly poison) on the skin, which was clear, colorless, and tasteless. Spider B seemed of the three the most willing to bite; hence, it was placed on the inside of the basal joint of the third finger of the left hand. It bit; however, the fangs were inserted but slightly into the skin, producing a slight sharp pain. This was at 11:15 A. M. At 11:20, I observed clearly visible pulsations at the place of the bite. 11:35. The pain is faintly perceptible at times. 12:25 P. M.: The pain is still felt at intervals. 2:10. No longer any sensation from the bite. During the afternoon, excessive perspiration was observed in an area of the size of a penny around the punctures.

On the following day, July 10, the test was repeated. Spider C was used; it bit as soon as placed, again on the inner surface of the basal joint of the third finger of the left hand. The fangs were allowed to remain inserted for about five seconds, and during this time the pain which when the fangs entered was rather faint, increased rapidly, presumably produced by the poison rather than by the fangs. The results, from the point of view of the investigator, were all that could

be desired. From the point of view of the one investigated, they were more than seemed desirable. The following account is a record of the symptoms and sequence of events as I observed them.

8:25 A. M. Spider bites as soon as placed, and the pain which at first is very faint, increases rapidly. When spider is removed, the pain keeps on growing, a sharp piercing sensation.

8:32. The place where the bite took place is whitish, like after a bee sting. Pain somewhat more dull, but decidedly present. Area of one inch in diameter around bite is very red, a slight swelling has appeared.

8:45. Pain about the same, not very marked. Swelling increased slightly, redness more intense, and spreading. A dull aching in tendons of arm pits.

9:15. Red area covered with small drops of perspiration, redness spreads over entire finger.

9:18. Aching in shoulder, lame feeling in entire arm.

10:25. Pain in finger rather severe, a burning sensation, extending over most of the hand, the red area now very red, sweating profusely. Pain in shoulder varies, strong at times. Some aching in muscles of chest.

10:50. Aching in chest, also to slight extent in hips.

11:00. Put absorbent cotton soaked in 10% NH_4OH on finger; does not seem to give much relief.

11:10. Pain in chest more marked, legs ache too.

12:20 P. M. Pain in hips rather severe. Chest feels cramped, breathing and speech are spasmodic. The doctor* advises that I go to bed.

12:30. Went to bed, and am sweating profusely.

1:30. The muscles of the legs, above the knee, begin to ache.

4:30. Pain in finger very severe, aching pain in chest and hips. Nervous tremor, present since noon, is more noticeable now. Applied $KMNO_4$ solution to finger.

5:15. Arrived at the hospital, and went to bed.

6:25. Took 2 Na Br pills, and a cup of coffee. The coffee comes back after a few minutes. Pain in Maximus gluteus and adjoining muscles very severe, breathing somewhat difficult; this and speech irregular and forced.

7:00. A hot bath; stayed in tub at least 30 minutes. Kept hand with dressing ($KMNO_4$) out of water. The diaphragm is entirely relieved; breathing and speech are normal. The pain in the muscles of the hips is temporarily relieved.

7:30. An electric oven was placed over the hand. The $KMNO_4$ solution is renewed from time to time. The heat increases the pain in the hand.

The oven was left on for about 2 hours. After that, it was taken off for an hour and then kept on for an hour till 4:00 A. M. Then I rebelled. The oven was taken away, and I removed the dressing.

July 11.—6:00 A. M. Pain in legs and hips very severe. Because of this pain all thru the night, I did not lie still for longer than about 30 seconds at a time.

8:00. A strip of pimples 1½ to 2 inches wide, and extending from the third finger on the outside of the hand, to the elbow, has appeared. (I felt this strip as wet and sticky last night.) Drank some coffee and retained it.

8:30. A hot bath. The left hand was kept in hot water for about 30 minutes. Pain relieved everywhere, including the hand. (There was scarcely any pain anywhere for a half an hour later; it gradually increased, but did not reach the degree it maintained throughout the night. The change in the hand was very marked. The hot water gave the first relief here.)

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*Dr. E. F. Ellis, of Fayetteville, was my physician and rendered very valuable service. The various forms of treatment tried, including the hot bath, were suggested by him. I am especially grateful for his genuine interest and sympathy. He has read the manuscript and has added to it some of his impressions regarding the symptoms and the behavior of the patient.

9:00. The doctor says I look cyanotic in the face.

12:55. Slept about 10 minutes, feel decidedly better. The pain in the hand is relatively slight; a marked aching in knees and toes.

3:10. Improvement very perceptible, slept again between 2 and 3.

5:30. Oyster soup; my first attempt to eat.

7:00. Hot bath.

July 12.—5:50 A. M. Slept for short periods, much troubled with delirium. As soon as I fell asleep, I would be frantically and in an utterly aimless fashion working with spiders. A slight headache; the pain in finger and elsewhere is relatively slight.

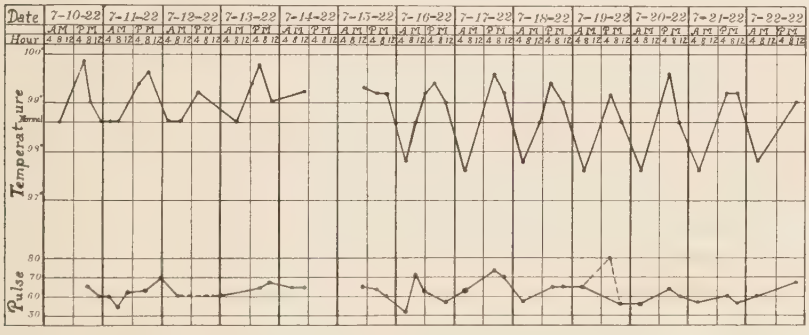
7:50. An aching pain in left hand, spasmodic; do not remember noticing it before.

8:30. Dull, not severe, headache since daylight; may be due to lack of nourishment. Have been reading in bed. This seemed impossible yesterday.

10:00. Hot bath.

6:30 P. M. Spent most of the day reading. Another hot bath.

8:30. Sat up from 7:30, with feet wrapped in blanket. Did not attempt this before because of a tendency to faint which troubled me when going to and from baths.



July 13.—5:20 A. M. Slept thru a large part of the night; some dreaming of innocent affairs.

11:30. Left the hospital.

1:30. Went to the office and tried to work. I realized that I had not quite recovered, felt wretched—neither sick nor well. Another hot bath in the evening (and again on the evening of the following day).

Beginning with the morning of July 14, I was practically normal so far as general feeling was concerned. There was still a slight pain in my left hand. On the following day this developed into a persistent itching which lasted for several days.

The body temperature, as is seen from the accompanying chart, continued to fluctuate for more than a month. The record on the chart is for thirteen days, July 10 to 22. After that the variation in temperature gradually became less. The fluctuation, whatever it may be due to, was not sufficient to bring with it any perceptible sensations. I felt as if in perfect health, had very good appetite, slept well, and had no particular indisposition to work.

Referring briefly to some of the general effects of the case, I would say that the sharp pain in the finger, or rather in the left hand, was the most prominent feature. Very nearly as unpleasant was the aching pain which was most violent in the thick muscles of the lower part of the back, and present in almost all the muscles of the shoulders, chest, and legs. There was no marked tendency towards profuse perspiration. I sweated heavily only when I first went to bed, and later after each one of the hot baths. I covered up well after these baths in order to bring about sweating, and I believe that it aided in recovery. There was no evidence of constipation. One dose of magnesium citrate brought fairly prompt results. On the day I left the hospital, I took a second dose, in order to facilitate recovery as much as possible.

With reference to treatment, I feel convinced that the hot water bath, as hot as the patient can endure, is by far the best measure that we tried. I would recommend a hot bath three to four times in twenty-four hours, even if the patient must have assistance to get to and from the bathroom. It is important that the region where the bite took place be kept in hot water during the bath. If the bite is on the hand or foot, it might be well to bathe in hot water much oftener. The treatment with $KMNO_4$ had no perceptible effect. The Na Br pills (I took about six of them at intervals of several hours) apparently did nothing to subdue the pain. The doctor did not use any morphine, for which I am very grateful. The body was thus enabled to throw off the poison with all possible expedition.

THE TOXICITY OF THE POISON

The evidence that has been presented here shows that the bite of the Black Widow is likely to cause decidedly unpleasant, and under certain circumstances, dangerous results. If mine were an average susceptibility, I would say that in cases of such susceptibility and an ordinary dose of the poison, the effects are not likely to be serious. However, there is no evidence as to my relative resistance to the poison. It may be unnusually high, and it may be fairly low. The place on the body where the bite takes place is an important factor. If a person is bitten where the skin is but slightly sensitive, as for instance on the neck, or on the scrotum,* the insertion of the fangs will scarcely be felt, and sufficient pain to attract the attention of the victim will develop only after a considerable amount of the poison has been injected. I believe that a large dose of the poison injected in the neck will cause a local pain so severe that the victim will scarcely retain consciousness.

* Dr. E. F. Ellis has told me of a case in which a black spider (presumably the Black Widow) bit a man on the scrotum, when the man was in a thoroughly inebriated condition. The patient suffered frightfully for several days. Recovery was slow and required several months.

NOTE BY DOCTOR ELLIS

The subjective symptoms in Mr. Baerg's case have been very graphically described by him. The objective symptoms would indicate, as observed by me, that there is a very marked phagocytosis locally around the area of the spider bite. The toxicity of the bite was such that the phagocytes very shortly offered no resistance to the systemic invasion of the poison. The poison in my opinion was partly transmitted through the blood stream and partly through the nerve trunk which in this case was the median nerve. Strange to say in this particular instance the patient had a marked vasomotor disturbance on the flexor side of the forearm, as was evidenced by a narrow strip something like an inch in width, extending up almost to the elbow in which there was very marked diaphoresis. This was present during the first twenty-four hours after the bite. The toxicity was also manifested by vasomotor changes in the lumbar muscles and muscles of the extremities, and in all the large joints of the body, as was shown by intermittent pains and symptoms similar to intermittent claudication. There seems also to be a disposition, on his part, to unload very slowly, by elimination, the products of poison. More so than is the case with bites of any of the snakes including the rattler that I have observed.

E. F. Ellis, M.D.

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LIMBERNECK OF FOWLS PRODUCED BY FLY LARVAE *

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That the eating by fowls of fly larvae will, under certain conditions, produce the disease commonly called limberneck, has been suspected by many poultry raisers for years. The practice of certain English game keepers of placing dead birds and animals in baskets hung in the trees so that the pheasants can feed upon the maggots as they fall to the ground without danger of swallowing any of the dead tissue and by so doing the pheasants are not adversely affected, strengthens the belief entertained by some people that it is the decaying tissue rather than the maggots that cause the malady. However, Dr. E. W. Saunders and his associates (1912, etc.) are of the opinion that the disease occurs among chickens and other avian species and that it is transmitted by the fly, *Lucilia caesar* L. They think this insect a necessary intermediate host and believe that the disease may occur in various quadrupeds and even man, the latter in the form of poliomyelitis.

An article by Wilkins and Dutcher dealing with experiments they conducted with the transmission of limberneck in fowls corroborated, in part, the conclusions of Dr. Saunders. They found *Lucilia caesar* larvae developed from eggs deposited on a limberneck carcass and on the carcass of a hog which died of paralysis to produce the disease in healthy fowls when fed to them while larvae developed from healthy beef were not infectious. Two other flies, *Musca domestica* L. and *Calliphora vomitoria* L., tested by them gave negative results.

These results and those published subsequently by Bengston (1922) have aroused considerable interest in this field and make desirable the publication of notes on experiments performed by the writer and his associates.

THE EXPERIMENTS SUMMARIZED

On Nov. 12, 1914, the head of a healthy calf was exposed to flies in a tree by J. D. Mitchell. Specimens of *Chrysomya macellaria* Fab., Lucilia spp., Sarcophaga spp., and *Musca domestica* L. were observed on the meat. November 16 and 28, a total of 800 full grown larvae were fed to 2 young roosters without any apparent ill effect. In the second feeding some of the meat was eaten with the larvae.

Butchers offal exposed on April 20, 1915, was freely blown by *C. macellaria* and 4 days later the mass, well filled with nearly grown maggots, was placed before 2 hens. They avoided the meat but ate freely of the larvae without sickening.

* Published by permission of the Chief of the Bureau of Entomology.

During the period of May 13 to 24, 2 hens from which feed had previously been withheld for 2 days had placed before them a putrid beef liver. They refused to eat the liver but greedily devoured about 200 fly larvae (species unknown) with which it had become infested. No injury was apparent. A similar test was made in September, 1916, when 3 pounds of putrid but uninfested beef liver was fed to a rooster and pullet after they had starved 38 hours. They ate it greedily and were not sick afterward.

On Sept. 13, 1915, a healthy rooster was killed and exposed to flies, *C. macellaria* predominating. It was then covered until September 19 when it was found to be swarming with larvae, many of which were fully grown. At this time two hens and a rooster were placed in the cage with the infested fowl on the ground. They ate heartily and during the succeeding 4 days scratched the carcass about, ate some of it as well as all larvae present but none of them showed any evidence of disease. A similar test was carried out later the same month with 2 half grown chickens but no sickness was produced.

On Aug. 18, 1916, Mr. H. P. Wood began some tests with material from a hen which had died in nature and from which other fowls apparently had picked up limberneck. Four hundred larvae about grown were removed from the carcass, washed in hydrant water and fed to a hen (No. 1). She appeared normal one and one-half hours later and 100 unwashed larvae were given her. The following morning she exhibited a typical case of limberneck and died that day before noon.

A rooster (No. 2; white leghorn) was drenched with 2 tablespoonsful of water in which the above larvae had been washed. He showed no ill effects subsequently. Another rooster (No. 3; R. I. Red) was fed a piece (probably 5 grams) of the flesh of the dead hen. The following morning he showed limberneck symptoms from which he recovered later. Rooster No. 2 was fed 50 full grown larvae taken directly from the carcass of the dead fowl on August 22, but did not sicken. This same fowl also ate, without ill effects, several hundred larvae on September 1. These were reared in the carcass of hen No. 1, which died of limberneck. The larvae, however, came up through about 8 inches of soil with which the carcass was covered.

Hen No. 4 (mixed breed) was fed about 600 fully grown larvae August 28, and 4 ounces, estimated at 3200, half grown maggots on August 30. These larvae were all from the carcass of hen No. 1 and had burrowed up through 8 inches of soil. Hen No. 5 (mixed breed) received 600 larvae of the same lot and neither of these hens sickened. On September 2, at 10 a. m., 100 full grown larvae were removed directly from the carcass of hen No. 1 and fed to hen No. 4. At the same time, 100 other larvae were removed and placed in a jar of earth until 5 p. m., when they were fed to hen No. 5. September 3, at

7:30 a. m., hen No. 4 had a clear case of limberneck from which she finally recovered while hen No. 5 showed but a slight diarrhea for 1 day.

Some of the *C. macellaria* adults bred from the carcass of hen No. 1 were placed on healthy beef and when the larvae reared from them were nearly full grown, 250 of them were fed to hen No. 5 on Sept. 11, 1916, at 2:30 p. m. The following morning this fowl exhibited a typical case of limberneck from which she died at 3 p. m. that day.

Hen No. 6 (brown leghorn) received 250 fully grown larvae of *C. macellaria* on Sept. 17, 1916. These were reared on healthy beef from flies collected with a net about a slaughter house. She refused larvae offered to her, subsequently, but on September 19, ate 5 larvae from the carcass of hen No. 5, which died of limberneck, and 4 larvae from the same source the following day. The same fowl on September 21, ate 125 larvae which were transplanted a few days before to healthy beef from the carcass of hen No. 5, when about half grown. She showed no symptoms of disease from any of these larvae.

Leghorn rooster No. 2 was fed 200 larvae taken directly from body of hen No. 1 and washed in a tumbler of water. He occupied some time in calling hens and probably some of the larvae escaped. No ill effects were noted.

SPECIES OF FLIES CONCERNED

In the experiments previously mentioned, Wilkins and Dutcher produced limberneck with larvae of *Lucilia caesar* L. reared in carcasses of paralyzed pigs or limberneck fowls. Dr. Saunders also associates this species with the transmission of certain paralytic diseases of animals, limberneck in fowls, and thinks it may carry anterior poliomyelitis of man. In our experiments with maggots reared on healthy beef refuse and liver the species fed to fowls were not determined accurately. The fact that *C. macellaria* was abundant practically insured the presence of that species in numbers in most of the tests. Specimens of *Lucilia sericata* Meig. and *L. caesar* and some Sarcophagid larvae were, no doubt, present in most instances.

In the tests with larvae from the hen found dead in nature, probably the larvae of *C. macellaria* predominated and some *Lucilia sericata* and Sarcophaga were present, as indicated by the fact that from a lot of these pupae, 85 specimens of *C. macellaria,* 1 *L. sericata* and 4 *Sarcophaga* sp. were reared.

The species of maggots from the carcass of limberneck hen No. 1 were also mixed, probably *C. macellaria* predominating, as indicated by the large number of that species bred out. In the case of limberneck produced in hen No. 5 the larvae used were *C. macellaria.*

SUGGESTIONS

While these experiments are of a preliminary character and are by no means conclusive, they are thought to be suggestive and the following points seem to be indicated:

Chrysomya macellaria larvae reared in carcasses of limberneck fowls and fed to healthy fowls are capable of producing limberneck. Other blow fly larvae may cause the disease under like conditions. The causative agency of limberneck may be carried by larvae reared in a limberneck carcass through the pupa and adult stage to the larvae of the next generation reared in beef. Meat from limberneck carcasses fed to fowls will cause limberneck. Larvae which have ceased feeding on infective carcasses, either on account of becoming full grown or through removal, and have more or less cleaned themselves by voiding infective material from the digestive tract and by burrowing through soil are less likely to produce limberneck when fed to fowls.

Washings from maggots fed on limberneck material are apparently not very toxic when given to fowls by mouth. A considerable amount of infected material is necessary to produce marked cases of limberneck in fowls. Certain breeds may be more resistant to limberneck than others. Blow fly larvae reared on putrid beef offal and liver are often non-injurious to fowls even though eaten in large numbers.

Putrid liver and beef offal and carcasses of healthy chickens are often non-toxic even though eaten by fowls in considerable amounts, and larvae of flies from such non-toxic material will not produce limberneck. In other words, it appears that there is a specific causative agency whether *Bacillus botulinus* or some related form, which when partaken of in quantity, either in meat or in fly larvae which have fed on such infested material, may produce limberneck.

The need of disposal by burning of all carcasses, especially those which have died of limberneck, is further emphasized.

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TELOSENTIS, A NEW GENUS OF ACANTHOCEPHALA FROM SOUTHERN EUROPE*

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Through the courtesy of Professor F. S. Monticelli, I have had the opportunity of examining some specimens of Acanthocephala previously identified as *Echinorhynchus lateralis* Molin from the intestine of *Atherina hepsetus*. A study of this material has demonstrated that the specimens in question belong to the subfamily Rhadinorhynchinae, though they cannot be included within any genus hitherto recognized. Consequently a new genus, Telosentis, is herewith proposed for the specimens and the new species, *Telosentis molini,* is designated as the genotype.

Telosentis, gen. nov.

With the characters of the subfamily Rhadinorhynchinae. Genital orifice subterminal. In both sexes the extremity of the body adjacent to the genital orifice bears a few scattered cuticular spines. Sexually mature in the intestine of fishes.

Telosentis molini, spec. nov.

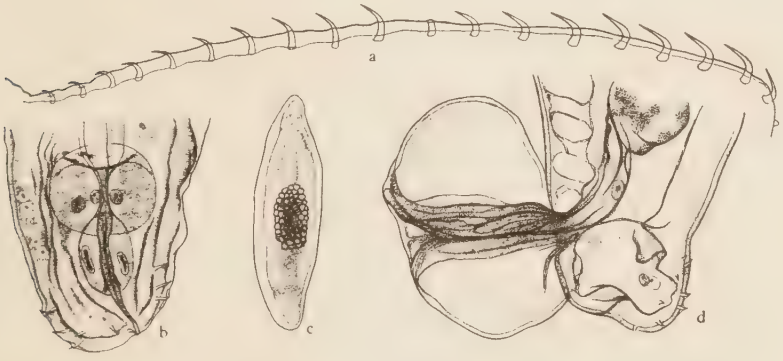
Body 6 to 8.6 mm. long. Proboscis cylindrical to club-shaped; 1.14 to 1.3 mm. long by 0.15 to 0.19 mm. in diameter; bearing 12 longitudinal rows of about 20 hooks each; hooks near middle of proboscis 48 to 60μ long, those on the ventral surface slightly larger than those at the same level on the dorsal surface. Body spines about 24μ long. Proboscis receptacle about 2.2 mm. long, with the brain about one third the distance from its posterior extremity. Lemnisci approximately the same length as the receptacle. Embryos within body cavity of gravid female, 60 to 72μ long by 12 to 14μ in diameter. Type host, *Atherina hepsetus,* from Italy.

Cotypes, comprising both males and females, are deposited in the collection of the writer at Urbana, Illinois.

It seems highly probable that *Echinorhynchus lateralis* Molin (1858) may be a synonym of *Telosentis molini.* However, this older specific name is not available because the same specific name had been used earlier (1851) by Leidy in describing an entirely different species from

* Contributions from the Zoological Laboratory of the University of Illinois, No. 211.

a North American host and as a consequence Molin's specific name could never be recognized as valid. In the literature there are various references to the occurrence of *"Echinorhynchus lateralis* Molin," but the descriptions and other data accompanying these references render the determination of the specific identity of the materials under consideration hopeless. In the publications of Stossich, Hamann, Barbagallo and Drago, Porta, and Parona, *"Echinorhynchus lateralis* Molin" has been recorded from the following host species: *Belone acus, B. vulgaris, Labrax lupus, Gobius jozo, Exocoetus volitans,* and *Anguilla vulgaris.* All of these records are from localities adjoining the Mediterranean and the Adriatic. Whether these references all pertain to a single species or to several species within a common genus cannot be determined at the present time.



Telosentis molini

Figure *a,* profile of dorsal surface of proposcis showing single longitudinal row of hooks (× about 100); *b,* posterior extremity of female (× about 225) showing characteristic cuticular spines; *c,* embryo from body cavity of gravid female (× about 560); d, posterior extremity of male with copulatory bursa extruded, showing cuticular spines (× about 100).

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NOTE ON DEGENERATING CESTODE CYSTS IN MACKEREL*

Edwin Linton

The encysted stage of cestodes which belong to the Tetrarhynchidae is of very common occurrence in many kinds of marine fishes. In a few species they are not infrequently found in the flesh. Usually, however, their resting place is in the viscera, either on the mesentery, or under the serous coat, or embedded in the walls of the alimentary canal. In the latter case their favorite lodgement is in the submucosa. It is a well known fact that the adult stage of cestodes is as a rule limited to a very few hosts while the encysted stage enjoys a wide range. For example, the cestode here considered, *Rhynchobothrium imparispine,* has for its final host, preferably, some species of skate belonging to the genus Raja altho it is occasionally found in other selachian hosts. In the encysted stage it has been found in a large number of species of marine teleosts. My notes show identifications of the larval stage in at least thirty-five species of teleosts in the Woods Hole region.

Among its intermediate hosts the larva of this cestode finds some more tolerant than others. Thus in the whiting *(Merluccius bilinearis),* and the hakes (Phycis) it was found frequently with well developed and active scoleces. The goosefish *(Lophius piscatorius)* is also tolerant of the encysted stage of this cestode. In the goosefish, cysts of relatively large size are quite common on the viscera. For example, while a diameter of 5 mm. is the maximum size of the cyst of *R. imparispine* in most intermediate hosts, I have found cysts in the goosefish which measured as much as 22 mm. in diameter. The scoleces obtained from these large cysts were no larger than those from cysts 5 mm., or less, in diameter. The presence of these large cysts in the goosefish is not due to any physiological peculiarity of the tissues of the host, but rather to the fact that the goosefish grows to a large size, and has, consequently a long lease of life, so that entozoa, once they are lodged in the body cavity, remain there indefinitely. Even in this favoring habitat cysts are often found in which the contents have become more or less completely calcified.

Among the fishes in which cysts of *R. imparispine* have been found, the mackerel *(Scomber scombrus)* may be cited as an example of host which appears to furnish an unkindly environment to the cysts.

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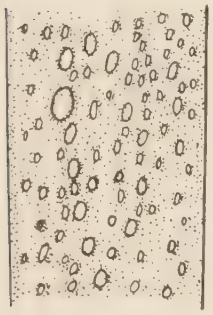
* Contribution from the U. S. Bureau of Fisheries Laboratory at Woods Hole, Mass., and the Medical Department of the University of Georgia, Augusta, Ga.

Following are extracts from notes on the occurrence of cestode cysts in the mackerel:

On July 1, 1912, the viscera of a good-sized mackerel were brought to the laboratory. There were a few small cysts on the serous coat of the stomach, and very many on the pyloric ceca. The largest cyst was little more than 2 mm. in diameter while the great majority were 0.5 mm., or less. Their color was whitish, but on account of the presence of a yellowish granular pigment on and about them, the general effect was that of yellowish seed-like bodies in the serous coat. A number of these cysts were crushed, and at first only granular material with minute oil globules, and slender, rod-like crystals were distinguished. The crystals appeared to lie in globular clusters, as if radiating from a common nucleus, which broke up into fascicled fragments when crushed. Upon further examination of crushed cysts, isolated hooks were found which suggested the hooks of *R. imparispine*. In one of the larger cysts clusters of hooks were found which showed the characteristic arrangement peculiar to that species. Later a scolex was found in one of the larger cysts; altho in good condition it showed no signs of life. The proboscides were fully developed, and bore the complement of hooks characteristic of the species.



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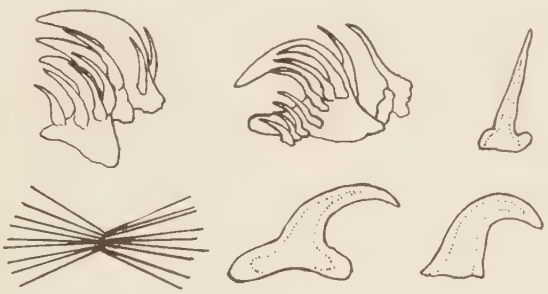

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Fig. 1.—Pyloric cecum of mackerel with cysts on serous coat; length of largest cyst about 2 mm.

Fig. 2.—Single hooks, groups of hooks and rod-shaped crystals, associated with calcified material and degenerate tissue in a small cyst from viscera of mackerel; length of longest hook 0.04 mm.

Some degeneration, however, appeared to have taken place in the bulbs; the retractile muscles, which could be traced in the sheathes, were not distinguishable in the bulbs and the outlines of the bulbs themselves were indistinguishable from the surrounding parenchyma at their posterior ends. Numerous calcareous bodies were scattered through the parenchyma, being most abundant in the bothria. Along side the anterior end of the scolex lay a closely packed mass of calcareous bodies, 1.36 by 1.22 mm. The calcareous bodies of which this mass was made up were similar to those in the scolex though many of them were larger. A concentric structure could be seen in many of them. They were enclosed in a definite membrane, which could be traced to a similar structure surrounding the scolex, thus serving to show that the mass of calcareous bodies was a remnant of the blasto-cyst. Fascicled, rod-like crystals were present, but not abundant. A cluster of small cysts was crushed and both rod-like crystals and calcareous material were found. While much of the contents of these cysts dissolved with brisk effervescence in weak hydrochloric acid, the slender, rod-like crystals were unaffected.

A few days later, on July 5, similar conditions were observed in some 12 mackerel which were examined on that date.

On June 27, 1913, numerous cysts on the viscera, and in the stomach wall, were found in 5 out of 12 fish. These cysts were as are recorded above but measured from 0.3 to 3.5 mm. in diameter.

On June 22, 1922, the viscera of a mackerel, 40 cm. long, were found to be thickly covered with cysts, from 1 mm. and less, to 5 mm. in diameter, many of them yellowish-brown. The submucosa of both stomach and intestine was crowded with cysts. A scolex in fresh water, somewhat compressed, measured 7 mm. in length.

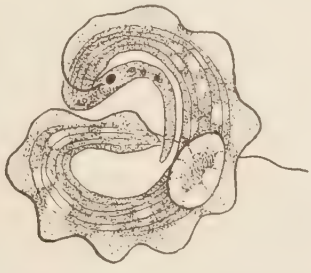
The following interpretation of this pathological condition is indicated: An infection produced by the invasion of onchospheres, of *R. imparispine* has been more or less successfully met by the phagocytes, or other resisting agents. In the case of the smaller cysts the invading parasite has been destroyed before the scolex had developed, at least, to the stage at which hooks appear. Those cysts in which isolated hooks, or groups of hooks, were seen represent cases where the battling forces of the host had succeeded in destroying the parasite only after a scolex had been formed. Furthermore, the cyst in which a moribund scolex was found presents a case in which the parasite held its own until a stage of development had been reached which might have developed into the adult, provided it had been eaten by the proper final host.

SKATE TRYPANOSOME FROM WOODS HOLE*

R. KUDO

This brief note is based upon the results of examination of blood smears of fish from the vicinity of Woods Hole, Mass. The number both in the individuals and species of fishes examined is small. It is however, presented here, since so far as I know, no record is to be found concerning the protozoan blood parasites of marine fishes from North America.

The material was collected during the summer of 1922 by Mr. Rodric Heffron of Chicago, to whom I am greatly indebted. One blood smear was made from each fish, air-dried and later stained with either Giemsa or Heidenhain's ironhematoxylin. The names and number of fishes examined are as follows: *Carcharhinus obscurus*, 1; *Isurus dekayi* (?), 1; *Raia ocellata*, 3; *R. erinacea*, 1; *Acanthocottus aeneus*, 2; *Brevoortia tyrannus*, 1; *Chilomycterus schoepfii*, 5; *Cyclopterus*



Trypanosoma raiae Laveran et Mesnil from *Raia ocellata*. × 3200.

lumpus, 3; *Gadus callarias*, 1; *Lophius piscatorius*, 2; *Menticirrhus saxatilis*, 2; *Merluccius bilinearis*, 1; *Palinurichthys perciformis*, 1; *Paralichthys dentatus*, 2; *P. oblongus*, 1; *Pollachius virens*, 2; *Pomolobus mediocris*, 1; *P. pseudoharengus*, 2; *Prionotus carolinus*, 5; *P. strigatus*, 3; *Rhombus tricanthus*, 1; *Scomber scombrus*, 2; *Stenotomus chrysops*, 2; *Tautoga onitis*, 2; *Tautogolabrus adspersus*, 3. Of these 50 fishes belonging to 25 species, only one winter skate (*Raia ocellata*) was found to be infected and that merely by a small number of trypanosomes.

The body of the trypanosome is coiled up as is usually the case with the large fish trypanosomes, the undulating membrane facing outside. The anterior end is pointed, while the posterior end is highly

*Contributions from the Zoological Laboratory of the University of Illinois, No. 216.

drawn out into a blunt tip. The undulating membrane is fairly well pronounced. The dense cytoplasm which is vacuolated at places, is finely and uniformly granulated except at the posterior portion of the body where a few coarse granules are noticed. Distinct myonemes run longitudinally, five or six being the usual numbers. The large oval nucleus, occupies the entire breadth of the body and possesses a large karyosome located at its center, from which radiate chromatic strands outward to the membrane. The blepharoplast is a small compact body surrounded by a narrow clear ring and is located some distance from the posterior tip of the body. The flagellum is comparatively short and hard to see. Dimensions: length of body, excluding the flagellum, 30 to 35μ; flagellum, 6 to 8μ long; largest breadth 2.3μ; broadest part of undulating membrane, 1.2μ; nucleus, 3 by 2.2μ; blepharoplast about 6μ from posterior to tip of body.

Of the three species of trypanosomes of European skates, *T. raiae* Laveran et Mesnil 1904, *T. giganteum* Neumann 1909 and *T. variabile* Neumann 1909, the form just described resembles closely the small form of *T. variabile* which was found by Neumann in the blood of *Raia punctata* at Naples, in the general form of the body and dimensions of body and nucleus. Minchin and Woodcock (1910) believe that *T. variabile* is identical with *T. raiae* which shows marked polymorphism. I consider the form from Woods Hole is identical with *Trypanosoma raiae* of European skates.

Contrary to expectations no haemogregarines were found.

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BOOK REVIEWS

PROTISTS AND DISEASE. VEGETABLE PROTISTS; ALGAE AND FUNGI. Including Chytridiineae; Various Plassomyxineae, the Causes of Molluscum Contagiosum, Smallpox, Syphilis, Cancer, and Hydrophobia; Together with the Mycetozoa and Allied Groups. By J. Jackson Clarke. William Wood and Company, New York, 1923. 225 pp., 61 figs.

In four preceding publications the author has used the general title Protozoa and Disease, but has been forced here to make the change indicated by reason of his conviction that the pathogenic organisms treated now are not Protozoa but allied to the Synchytriaceae. In his study of the cell inclusions that are common in lesions of molluscum contagiosum, cancer and other diseases, the author has reached the conviction that previous investigators have failed because they were "barking up the wrong tree" and that better success will attend the work of careful students of the lower plants to which, especially the Synchytriaceae and Olpidiaceae, he relates his present findings.

The author lays marked emphasis on the defects of the cell-theory as ordinarily conceived and is a stout protagonist of the view that nuclei may arise *de novo* from an amorphous basis. He believes he is able to demonstrate that non-nucleated phases play a regular and prominent part in the life history of the causal organisms in diseases such as those cited above and furnish the key to a satisfactory explanation of the maladies.

The thesis advanced and the studies reported in this volume carry one deeply into the field of little-known plant organisms and involve the assumption as facts of reports that often are unconfirmed and in some cases questioned by those most familiar with the organisms noted. The views advanced are interesting and will draw well desired attention to uncultivated and no doubt important fields. It would be venturesome to assert that all the author's theses will meet confirmation and one may safely assert that today at least many cytologists would hesitate to approve his discussions of chromidia and the nuclei of a protozoan which form the topic of his concluding chapter. The final paragraph may be cited as it illustrates well the author's views. He writes: "If the cell-theory is adjusted to amply proven facts it must read: New nuclei arise not only by division of pre-existing nuclei, but also by free nucleus-formation from chromidia or from plasson." The work deserves to be widely read and the problems it suggests should be exhaustively studied.

The addresses at the dedication ceremonies of the Peking Union Medical College together with the papers read at the medical conferences held on this occasion have just been issued in a single fine volume. It contains in addition to much other valuable material numerous items of especial interest to parasitologists to which only brief mention can be given here. Hookworm, plague, and chemotherapy in parasitic diseases are among the subjects of the special addresses; and parasitology in China and the Philippines is handled extensively in separate papers read at the conference of the department of pathology.

NEW HUMAN PARASITES

Agamofilaria streptocera Macfie and Corson, 1922.—In the skin of natives in the Gold Coast Macfie and Corson (Ann. Trop. Med., 16: 465; Dec. 30, 1922) found in 9 out of 24 cases examined for *Onchocerca volvulus* sheathless larvae which they maintain represent a new species. These larvae are noticeably slender, being 180 to 240μ long (average 215μ) and about 3μ broad. There is a row of ten to twelve nuclei in single file at the anterior end. The nerve ring is about 27% of the length from the anterior tip; the excretory cell about 34%, the G^1 cell about 69% and the anus about 86% from the same point. The tail is sharply crooked, abruptly pointed and marked by a clear area only 1μ long. These larvae were found only in the skin and in one necropsy as well as 22 out of 50 healthy individuals examined later in addition to the cases cited first.

Haemogregarina gallica Noc 1922.—This parasite was found in the blood of a 59 year old patient with pernicious anemia of the cryptogenetic type. The patient apparently had never been out of France. The study of his case was interrupted by his death soon after he came under observation. In several blood smears different stages were found including a crescentic form, a vermicular form, two division forms, and two merozoites. The crescentiform parasite was heteropolar, one of its extremities being terminated by a curved mucronate tip, the other by a bent tail-like process. The length of the body was 14.4μ, average width 4.5μ; the tail measured 5.4μ, giving the parasite a total length of 19.8μ. The protoplasm was reticulated, finely alveolar, surrounded by a simple membrane. Presence of a capsule uncertain but the parasite was apparently attached to a degenerated red blood cell by which it seemed to be inclosed. There were two vacuolated nuclei, submedian and preterminal, respectively, the first containing three chromatin masses, the second, one. The vermicular form measured 15 by 5μ, containing an oval chromatin granule, and presenting a small gregariniform process anteriorly, and a slender, straight prolongation posteriorly. The merozoites were curved, 5 to 6μ long by 1.5μ wide. Comparison with the descriptions of *Haemogregarina hominis* Krempf 1917 (from China), *H. inexpectata Roubaud* 1919 (from a Belgian woman who had been in the Congo), and *H. elliptica* Sergent, Sergent and Parrot 1922 (from Corsica) shows certain differences, although *H. gallica* is not very different from *H. inexpectata* (Bull. soc. path. exot., 15: 936-943, fig. 1).

Haemogregarina aequatorialis Nattan-Larrier, 1922.—Hemogregarines were found in blood smears from a Frenchman, 31 years old, returned from the French Congo. Malarial parasites and trypanosomes were also present. The hemogregarines usually showed the form of a plano-convex lens, homopolar, with rounded ends. Length 6 to 7.5μ, width 1.6 to 2.4μ. Cytoplasm contains a rather large number of chromatin granules, and possibly small pigment granules. Nucleus usually in middle near convex border of parasite, occupying a third, rarely a half, of the transverse diameter; rounded or oval, homogeneous or finely granular. In some cases the parasites are surrounded by a delicate refringent membrane. This species most nearly resembles, among species heretofore reported from man, *H. elliptica* Sergent, Sergent and Parrot 1922. (Bull. soc. path. exot., 15: 943-947).

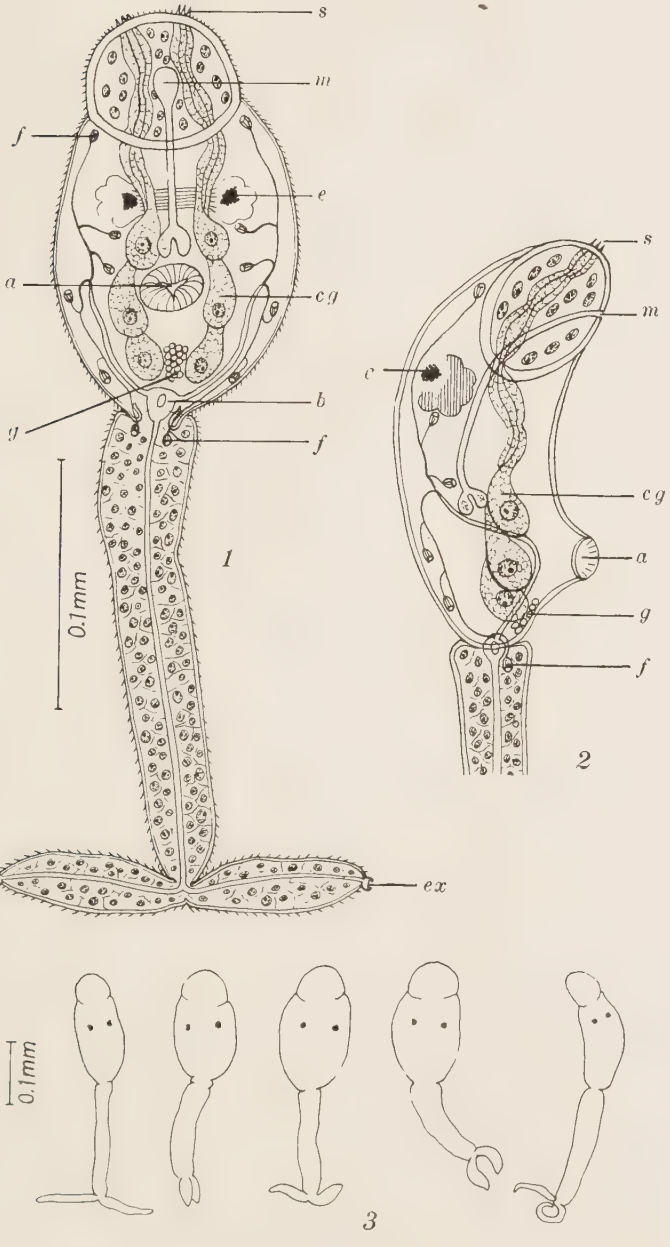

PLATE XIV

EXPLANATION OF PLATE XV

Fig. 4.—Freshly discharged egg in shell. × about 940.

Fig. 5.—Egg from feces diluted with 5% formalin, shell and vitelline membrane clearly shown. × about 940.

Fig. 6.—Free miracidium. × about 940.

Fig. 7.—Plump form of miracidium. × about 940.

Fig. 8.—Genital system of adult male. × about 170.

Fig. 9.—Genital system of adult female. × about 170.

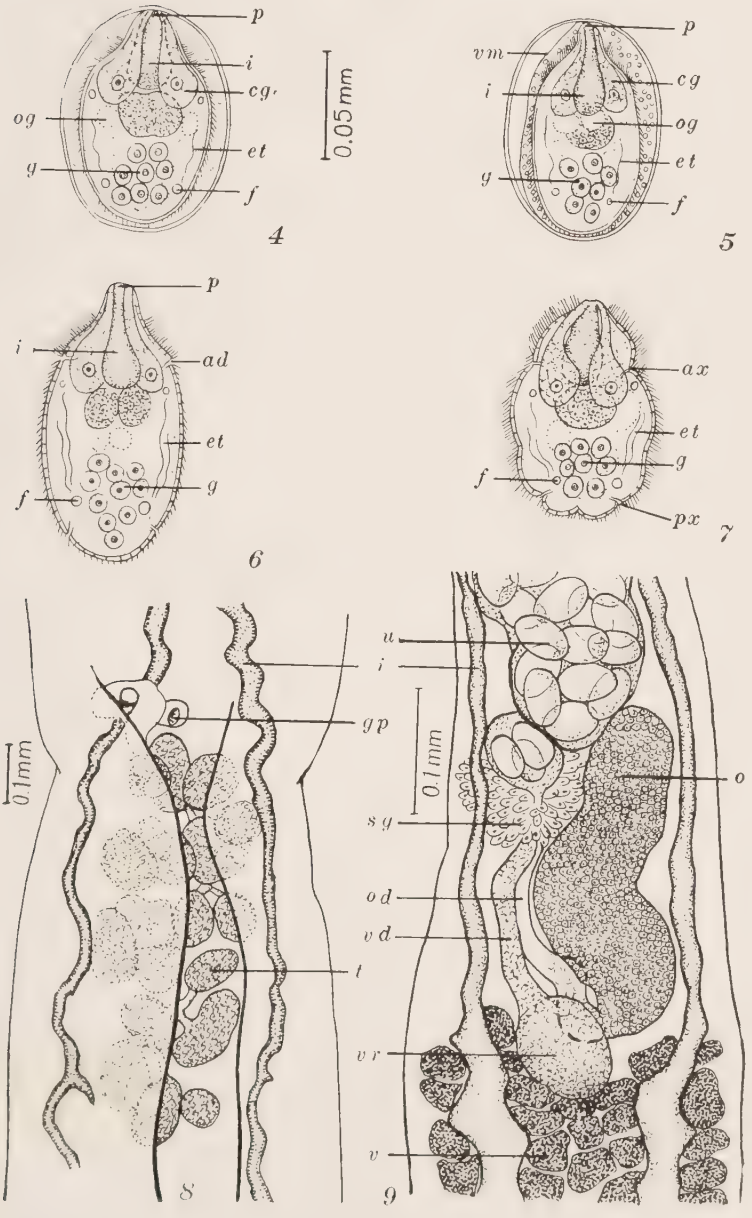

PLATE XV

EXPLANATION OF PLATE XVI

Fig. 10.—Egg in uterus near pore, just about to be deposited. × about 940.

Fig. 11.—Egg a little later in blood vessel. × about 940.

Fig. 12.—Adult male. × about 48.

Fig. 13.—Adult female. × about 48.

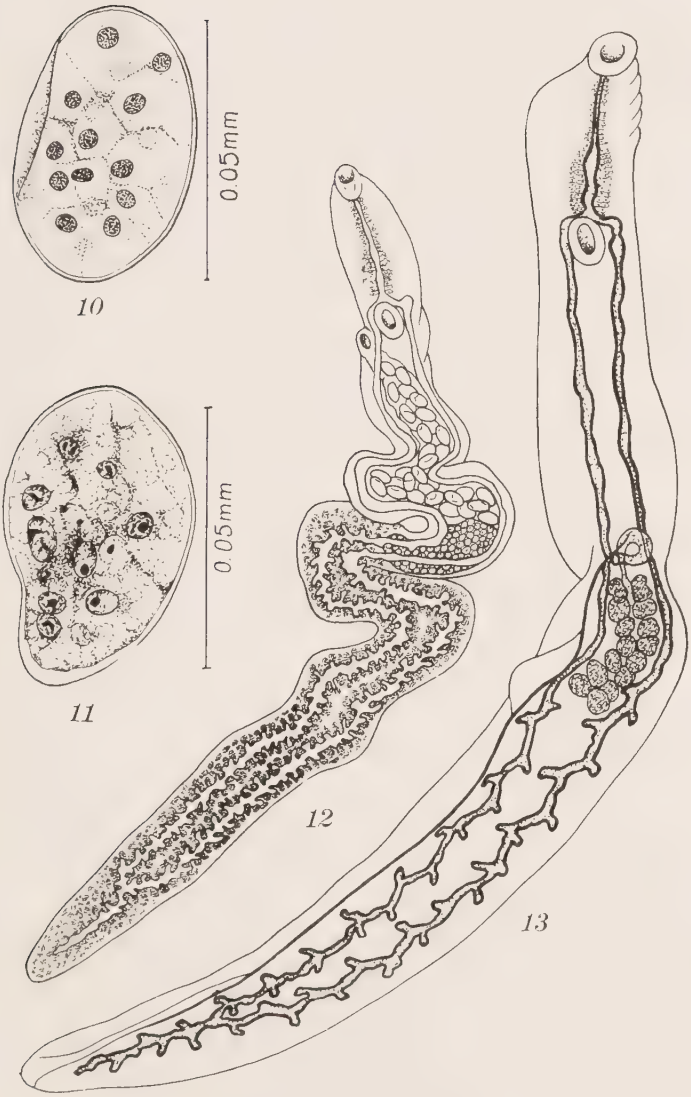

PLATE XVI

Explanation of Plate XVII

Figs. 14 and 17.—Male.

Figs. 18 and 22.—Male and female in copulation.

Fig. 23 and 25.—Female alone.

Fig. 14.—Cross section through anterior portion of body.

Fig. 15.—Cross section through constricted region of body.

Fig. 16.—Cross section through testes.

Fig. 17.—Cross section through posterior portion of body.

Fig. 18.—Cross section through anterior portions of male and female.

Fig. 19.—Cross section through testes and uterus.

Fig. 20.—Oblique section through testes, and uterus and ovary.

Fig. 21.—Cross section through posterior regions.

Fig. 22.—Longitudinal section through posterior regions.

Fig. 23.—Cross section through esophagus.

Fig. 24.—Cross section through uterus.

Fig. 25.—Longitudinal section through posterior portions.

Figs. 14-25.—× about 60.

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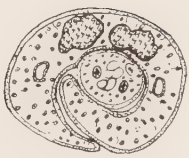

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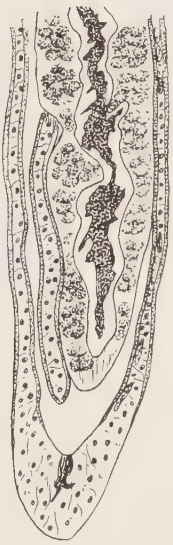

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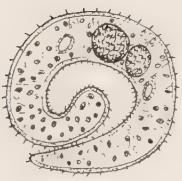

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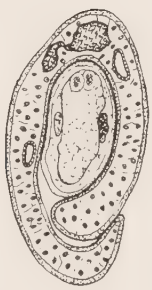

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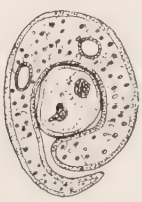

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PLATE XVII

Explanation of Plate XVIII

Figs. 26-33.—Series of developmental stages.

Fig. 26.—Very immature male from liver of mouse, twelve days after infection; fine granules of pigment scattered through body.

Fig. 27. —Very immature male, fourteen days after infection; ventral infolding of sides of body has begun in post-acetabular region.

Fig. 28.—Immature male from liver of mouse, eighteen days after infection; beginnings of testes are visible.

Fig. 29.—Nearly sexually mature male from liver of mouse, twenty-one days after infection; testes have become distinct, 12-16 in number; intestines are not united.

Fig. 30.—Nearly sexually mature female from liver of mouse, twenty-one days after infection.

Fig. 31.—Sexually mature male from liver of mouse, twenty-four days after infection; testes have become differentiated.

Fig. 32.—Sexually mature male from liver of mouse, thirty days after infection.

Fig. 33.—Sexually mature male, thirty days after infection, and nearly sexually mature female, twenty-one days after infection, in copulation; from mesenteric vein of mouse.

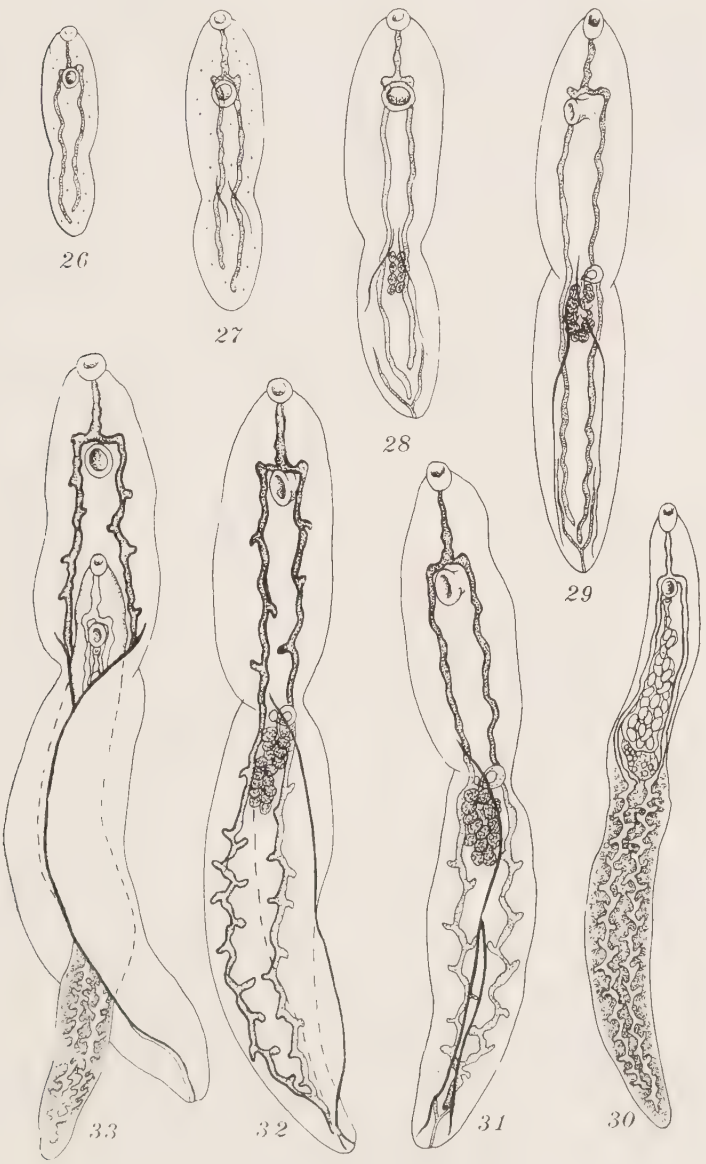


PLATE XVIII

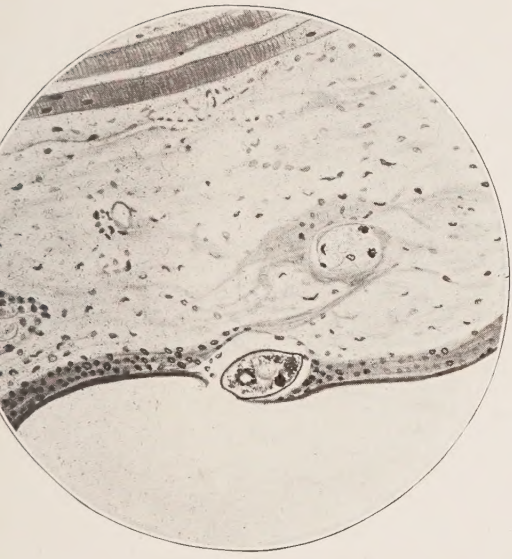
Explanation of Plate XIX

Figs. 34 and 35.—Sections through skin of tail of very young mouse which had been immersed for two hours in water containing large numbers of cercariae. × about 338.

Figs. 36-37.—Tubercle-like capsules around eggs of *Schistosomatium pathlocopticum* introduced into intralobular vessels through portal vein of mice infected with cercariae.

Fig. 36.—Proliferated epitheloid cells, giant cell, leukocytes, and commencing fibrosis around nearly resorbed egg; from liver of mouse, forty-five days after infection. × about 224.

Fig. 37.—Cell infiltration around degenerating eggs; from liver of mouse, thirty-four days after infection. × about 224.



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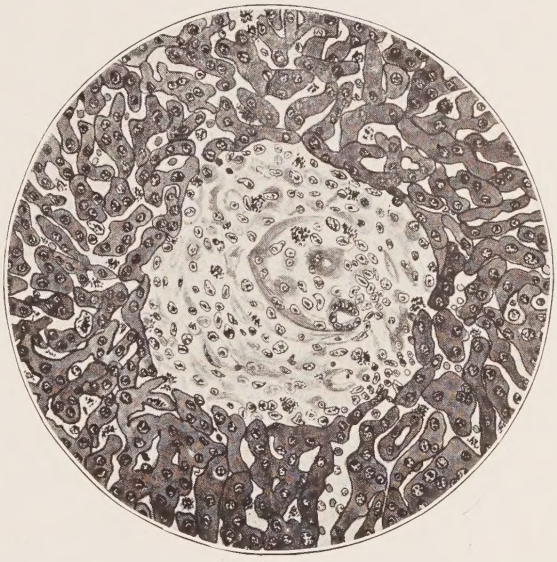

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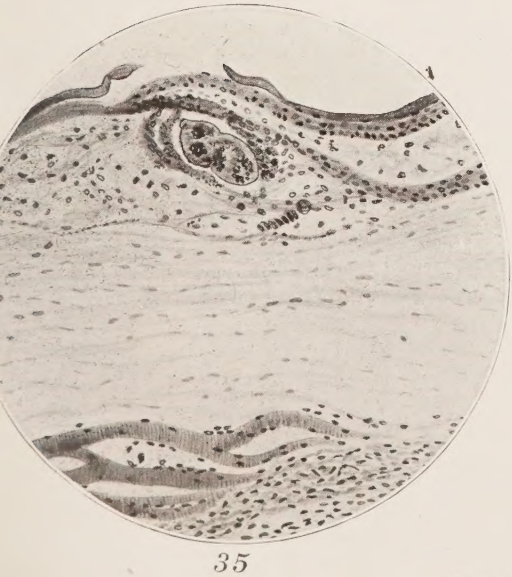

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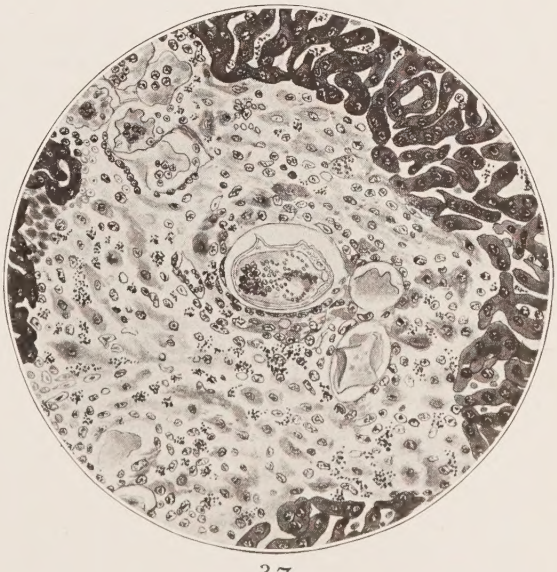

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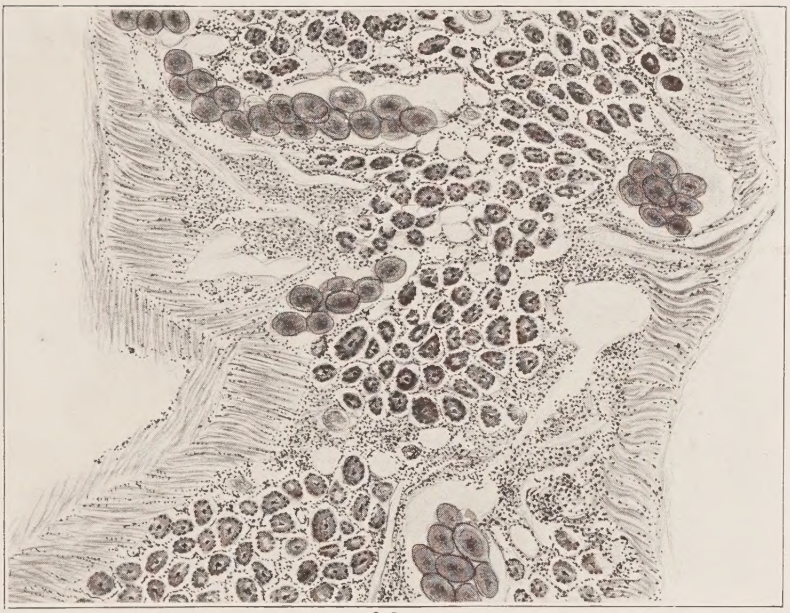
PLATE XIX

Explanation of Plate XX

Fig. 39.—Cross or oblique sections of adult worms, male and female; eggs sixth day after infection with cercariae; stained with Unna's alkaline methylene blue and eosin. × about 80.

Fig. 39.—Cross or oblique sections of adult worms, male and female; eggs deposited in portal vein of mouse which died on twenty-eighth day after infection with cercariae. Giemsa staining. × about 80.



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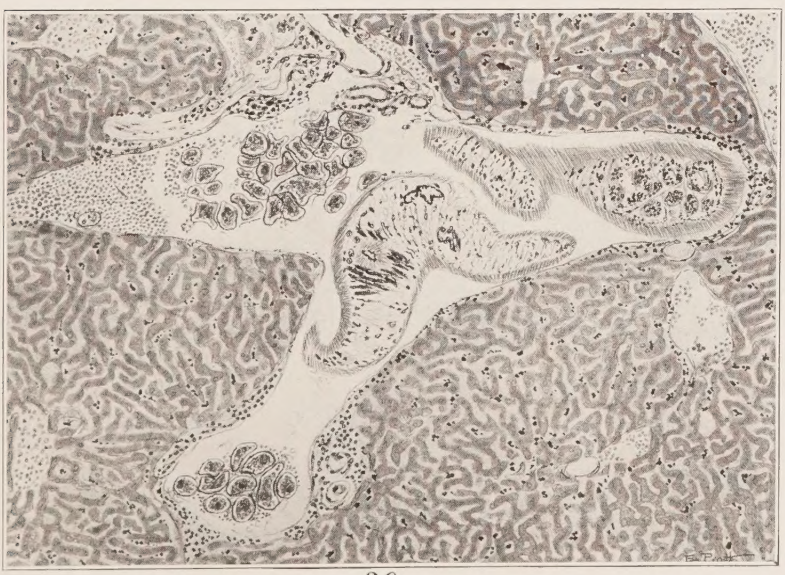

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PLATE XX